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FEBRUARY 1955

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AUSTRALASIAN ANNALS OF MEDICINE

Journal of The Royal Australasian College of Physicians

VOLUME IV, 1955, NUMBERS 1 - 4

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CONTENTS

Volume 4, Number 1

Volume 4, Number 2

CONTENTS—*Continued*

VOLUME 4, NUMBER 3

VOLUME 4, NUMBER 4

VOLUME 4 FEBRUARY 1955 NUMBER 1

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CONTENTS

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Editorial communications should be addressed to the Honorary Secretary of the Editorial Committee, The Royal Australasian College of Physicians, 145 Macquarie Street, Sydney.

The journal is published quarterly in February, May, August, and November. The subscription rate will be three pounds per annum, payable in advance to The Royal Australasian College of Physicians, 145 Macquarie Street, Sydney. Advertising rates may be obtained on application to Australasian Medical Publishing Company Limited, Seamer and Arundel Streets, Glebe, Sydney.

AUSTRALASIAN ANNALS OF MEDICINE

VOLUME 4 FEBRUARY, 1955 NUMBER 1

PORPHYRINS IN HEALTH AND DISEASE[1]

R. LEMBERG

From the Institute of Medical Research, The Royal North Shore Hospital of Sydney

IN 1841, Scherer transformed hæmoglobin into a purple pigment by the action of concentrated sulphuric acid. Hoppe-Seyler studied this pigment in 1871 and called it hæmatoporphyrin. He was also the first to obtain porphyrins from chlorophyll by the action of alkali, porphyrins which were later extensively studied by Willstätter.

Porphyrin in abnormal urine was first observed by Baumstark in 1874, and studied in the early 1890's by Salkowsky, Garrod and Saillet. Although Saillet noticed spectroscopic differences between his "urospectrin" and hæmatoporphyrin, the two were wrongly assumed to be identical. Hæmatoporphyrin is now recognized as an alteration product of protoporphyrin, the latter being the prosthetic group of hæmoglobin; hæmatoporphyrin arises from protoporphyrin by addition of water molecules to unsaturated vinyl side chains. The urinary porphyrins are again different, and consist of coproporphyrins with four and uroporphyrins with eight carboxyl groups, instead of the two found in protoporphyrin.

The nucleus (Figure I), porphin, is a macrocyclic ring consisting of four pyrroles condensed by four methine (−CH=) groups. This structure of the porphyrins was first proposed by Küster in 1913, on the basis of studies on the products of oxidative and reductive scission, and after a long wrangle, finally accepted by H. Fischer and confirmed by his classical synthesis of hæmin in 1929. Present chemical knowledge in this field is largely based on the immense analytical and synthetic work of Fischer. But the great merits of Schumm, who first demonstrated the distinction between various porphyrins by careful spectroscopic observations, have been obscured by the superseding of his nomenclature by that of Fischer.

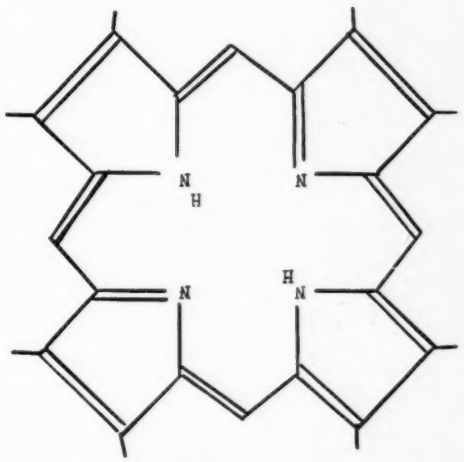

FIGURE I

The porphin ring. The eight β-positions on the four pyrrole rings (indicated by short lines) carry the side chains distinguishing the individual porphyrins. (These are set out in the text)

Porphyrins are derivatives of porphin in which eight β-positions on the pyrrole rings are substituted by various side chains, as follows:

Proto	$4CH_3$	$2CH_2.CH_2.CO_2H$	$2CH=CH_2$
Meso	$4CH_3$	$2CH_2.CH_2.CO_2H$	$2C_2H_5$
Hæmato	$4CH_3$	$2CH_2.CH_2.CO_2H$	$2CHOHCH_3$
Deutero	$4CH_3$	$2CH_2.CH_2.CO_2H$	2H
Spirographis	$4CH_3$	$2CH_2.CH_2.CO_2H$	$1CH=CH_2$; 1CHO
Ætio	$4CH_3$	$4C_2H_5$	
Copro	$4CH_3$	$4CH_2.CH_2.CO_2H$	
Uro	$4CH_2.CO_2H$	$4CH_2.CH_2.CO_2H$	

[1] Received on September 28, 1954. A post-graduate lecture, delivered under the auspices of the Post-Graduate Committee in Medicine in the University of Sydney on August 24, 1954.

Protoporphyrin has four methyl groups, two vinyl groups and two propionic acid side chains. Coproporphyrins have four methyl and four propionic acid side chains; uroporphyrins four acetic and four propionic acid side chains. The porphyrin *a* of cytochrome oxidase, or the *Atmungsferment*, has a formyl and a long alkyl side chain. Finally, in the chlorophylls an additional isocyclic ring is present which is produced by oxidative condensation of a propionic acid side chain with a methine bridge (Granick, 1948, 1951).

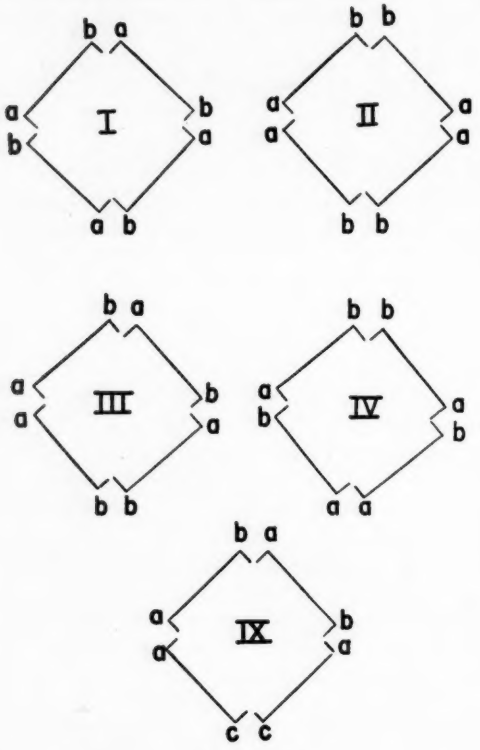

FIGURE II

Porphyrin isomers; (i) to (iv): the four isomers of porphyrins having the same two types of side chains (a and b) on all four pyrrole rings (for example, coproporphyrins and uroporphyrins); (ix): the naturally occurring of the 15 isomers of protoporphyrin and related porphyrins, which have three types of side chains (in protoporphyrin a=methyl, b=ethyl, c=propionic acid radical)

According to the arrangement of these side chains on the nucleus, several isomerides are possible. In the simplest case, with two different types of side chains, as in the coproporphyrins and uroporphyrins, four isomerides are possible (Figure II, (i) to (iv)), of which only two have so far been found in nature. These are the unsymmetrically substituted type III, and the centrosymmetrically substituted type I. Protoporphyrin, having three different types of side chains, can theoretically exist in 15 isomeric forms (Fischer, 1937); the natural type IX protoporphyrin (Figure II, (ix)), the prosthetic group not only of hæmoglobin, but also of myoglobins, some cytochromes, catalase and some peroxidases, is derived from type III. So are all the functionally important compounds in nature, not only the hæmoglobins and hæmatin enzymes, but also the chlorophylls and bile pigments.

Chlorophylls are magnesium complexes of dihydroporphyrins or tetrahydroporphyrins, and bile pigments contain a porphin ring opened by oxidative removal of one of the methine bridges. All these compounds are comprised under the name of tetrapyrroles.

FREE PORPHYRINS

Free porphyrins are widespread in nature, but usually in very small amounts (microgrammes), not only in vertebrates, but also in yeast and other microorganisms, in leguminous root nodules, in worms, in mussel shells and in coelenterates. Larger amounts (up to hundreds of milligrammes) are found only in certain diseases of " inborn error of metabolism " (Garrod, 1923), the porphyrias, and physiologically in the American ground squirrel (Turner, 1937). Coproporphyrins and uroporphyrins of both types, I and III, have been isolated. The red pigment of the feathers of the Turaco bird is a copper complex of uroporphyrin III. Finally recent chromatographic studies have revealed the presence in small amounts of porphyrins with 5-7 carboxyls standing between coproporphyrins and uroporphyrins (compare the recent review of Lemberg, 1954).

The isolation of individual porphyrins, particularly isomerides, from a complicated mixture is a difficult task, necessitating the conjoined application of a great variety of methods, study of the melting point of esters, chromatography, infra-red spectra, crystallographic properties and X-ray powder diagrams; the task is far from completed and surprises are not impossible.

Free protoporphyrin and still smaller amounts of coproporphyrin are present in red cells. Small amounts of coproporphyrin occur in the white matter of brain (below 10 microgrammes per 100 grammes), particularly in sensory nerves, and in the spinal cord. The

daily excretion of coproporphyrins in the urine amounts to about 100 to 250 microgrammes, in the fæces to 200 to 500 microgrammes. Uroporphyrins occur in still smaller quantities and are excreted only in the urine. The normal excretion is about 20 microgrammes per day (Lockwood, 1953*a*), and crystalline uroporphyrin ester (I and some III) has been isolated from normal urine (Lockwood, 1954). Uroporphyrin is deposited in fœtal bones, and in adult bones is still found in the ossicles of the inner ear.

If these values for porphyrin excretion are compared with the amounts of bile pigment formed daily by hæmoglobin breakdown (about 250 milligrammes per day), it is seen that the bile pigment formation is about 1000 times larger than the normal porphyrin excretion. It is only the very characteristic fluorescence and light absorption of the porphyrins which has made it possible to study such small amounts with considerable accuracy.

PORPHYRIN METAL COMPLEXES

An entirely wrong picture of the role and quantity of porphyrin synthesis in nature is obtained, if only the free porphyrins are taken into consideration. It is only a small percentage which escapes combination with metal—for example, iron—and incorporation into such molecules as hæmoglobin. Actually the amount of porphyrin synthesis on earth is immense. Chlorophyll forms about 0·5% of green leaves. A human adult produces more than 80 grammes of protoporphyrin annually, so that the production of mankind alone measures more than 160,000 tons *per annum*. The potential of this synthesis is far higher, a fact on which depends the possibility of having blood donors and blood banks. The amount of hæmatin enzymes, which are usually present in small concentration, must in aggregate also be very high on account of their almost ubiquitous occurrence in living cells.

As iron complexes (hæm compounds) and magnesium complexes (chlorophylls), porphyrin derivatives are essential for the three most fundamental biological processes, photosynthesis, cellular respiration and oxygen carriage and storage. The greater quantity of hæmoglobin in man and vertebrates does not signify that it is the most important biological compound. Actually, the hæmatin enzymes, particularly the cytochromes, on which cellular respiration depends, are of more general fundamental importance. The oxygen-carrying function of hæmoglobin is essential only for more bulky and complex animals such as man, in whom diffusion is insufficient to bring enough oxygen to the cells. Though hæmoglobin is found in most vertebrates, a few fishes have recently been found lacking it. The data on porphyrin and hæm metabolism are still very lopsided, in that very little is known about the metabolism of hæm enzymes, and also of myoglobins.

PORPHYRIN SYNTHESIS

The great advance of chemical knowledge on the tetrapyrroles in the first quarter of the century has been followed in the second quarter by an equally great advance of knowledge in hæmoglobin metabolism and porphyrin biosynthesis. The latter was due to two developments, an entire reevaluation of the interrelationships of porphyrins (compare Lemberg and Legge, 1949) and an almost accidental discovery by the use of isotope-tracer methods (Shemin and Rittenberg, 1946). The confluence of these two streams has led to an amazingly rapid development in the last eight years. The study of pathological processes has greatly contributed to this development, and sooner or later knowledge of the chemical mechanism of the normal physiological process will, in turn, provide possibilities for understanding the ætiology of the porphyrias, and perhaps means of curing them.

With the exception of a few organisms which require protoporphyrin as a growth substance (most of the trypanosomes, some flagellates and bacteria of the *Hæmophilus influenzæ* type), the process of porphyrin synthesis, far from easy for the organic chemist, is readily carried out by the cell. Porphyrin synthesis rarely becomes the limiting factor, and this is the reason why attempts to obtain insight into the mode of porphyrin synthesis by the study of factors necessary for hæmatopoiesis have failed. Only a few of the many factors necessary for hæmatopoiesis, the absence of which causes anæmia, affect hæmoglobin synthesis directly; most act on erythropoiesis, red cell maturation or red cell stability, and even when hæmoglobin synthesis is affected, it is iron metabolism and globin formation rather than porphyrin synthesis. Of the vitamins, only pyridoxine and pantothenic acid appear to be involved in porphyrin synthesis.

In view of the quantitative preponderance of protoporphyrin bound in hæmoglobin and the very small amounts of coproporphyrins and uroporphyrins, it was perhaps natural that Fischer (1937) assumed the latter to be breakdown products of protoporphyrin. It is a very common error to believe that the quantitatively

insignificant, or the rare, is secondary and of minor importance. In the pyrrole field, more than one example of this attitude having delayed scientific progress can be found. The stone that the builders rejected has, indeed, often become the corner stone.

The first breach in the accepted theory was the demonstration that porphyrins are not intermediates in the breakdown of hæm to bile pigments (Figure III). Lemberg (1935, 1937; also Lemberg and Legge, 1949) showed that the oxidative scission of the porphin ring occurs while the porphyrin is still combined with iron and protein, and that porphyrins are therefore not formed during the formation of bile pigments

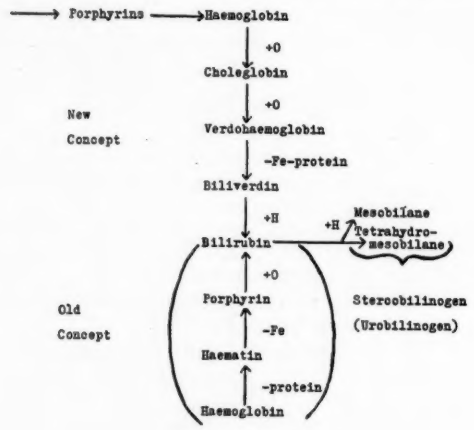

FIGURE III

The new and the old concepts of hæmoglobin breakdown. In the new concept porphyrins are not intermediates in the breakdown of hæmoglobin, but are intermediates or side-lines in hæmoglobin formation

from hæmoglobin. Biliverdin, the key substance and primary bile pigment, is rarely predominant in man and mammals, and had thus been considered as a secondary alteration product of bilirubin.

Then, studies of porphyrin excretion in pathological conditions, by Dobriner, Watson and others, showed that increased porphyrin excretion is far more closely connected with increased hæmatopoiesis than with increased hæmoglobin breakdown (Dobriner and Rhoads, 1940; Watson and Larson, 1947; Rimington, 1952; compare also Lemberg and Legge, 1949). Thus, after administration of phenylhydrazine, the increase of porphyrin excretion occurs, not when bile pigment formation is maximal, but later when hæmatopoiesis and reticulocyte formation are increased. Moreover, in conditions accompanied by hæmolysis—for example, in congenital porphyria—the free porphyrins excreted, uroporphyrins and coproporphyrins, are of type I, which cannot possibly be derived from type III protoporphyrin of hæmoglobin. This led Rimington (1938) and Dobriner and Rhoads (1940) to the theory that the synthesis of porphyrins is not fully specific, that type I porphyrins arise as by-products of this synthesis, and that they are excreted as unsuitable for incorporation into hæmatin compounds. However, the full significance of this became clear only later, when the idea that coproporphyrins and uroporphyrins were formed from protoporphyrin by carboxylation processes was abandoned.

Van den Bergh *et alii* (1932) had claimed that protoporphyrin could be converted into coproporphyrin III by the rabbit liver, when the liver was perfused with defibrinated blood with protoporphyrin added. However, this could not be confirmed by Watson, Pass and Schwartz (1941), and the conversion of protoporphyrin to coproporphyrin has now been disproved by isotope experiments (Grinstein, Kamen and Moore, 1948). Turner (1937), who found the normal porphyria of the fox squirrel, was the only one to postulate uroporphyrin as the primary porphyrin at that time, but there was no direct evidence, and this stone was again rejected. From a biochemical point of view, the assumed carboxylation of protoporphyrin appears far less likely than the opposite process (Figure IV)—that is,

PROTO		COPRO		URO
2 .CH=CH_2	⟵	2 .CH_2.CH_2.CO_2H		2 .CH_2.CH_2.CO_2H
2 .CH_2.CH_2CO_2H		2 .CH_2.CH_2.CO_2H		2 .CH_2.CH_2.CO_2H
4 .CH_3		4 .CH_3	⟵	4 .CH_2.CO_2H

FIGURE IV

Porphyrin synthesis. Relation between porphyrins. The side chain alterations assumed in the new concepts are indicated by arrows. These reactions do not necessarily occur in the porphyrins themselves, but may occur in monopyrrolic or dipyrrolic precursors

decarboxylation of the acetic acid groups of uroporphyrin (or of a precursor of it) to the methyl groups of coproporphyrin, and oxidative decarboxylation of two of the four propionic side chains of coproporphyrin (or of a precursor) to the vinyl side chains of protoporphyrin.

In 1946, Rittenberg and co-workers (Shemin and Rittenberg, 1946; Ponticorvo, Rittenberg and Bloch, 1949) published their discoveries that N^{15} of glycine and deuterium from acetate were incorporated in the hæm of hæmoglobin by

nucleated birds' erythrocytes. From the facts discussed above, I realized that now the mode of porphyrin synthesis could be understood, when a monopyrrole with the side chains of uroporphyrin—that is, acetic and propionic acid groups—was accepted as the primary pyrrole. It is well known that acetic acid enters the tricarboxylic acid cycle and that it is converted *via* citric acid into α-ketoglutaric acid, a key substance of carbohydrate and

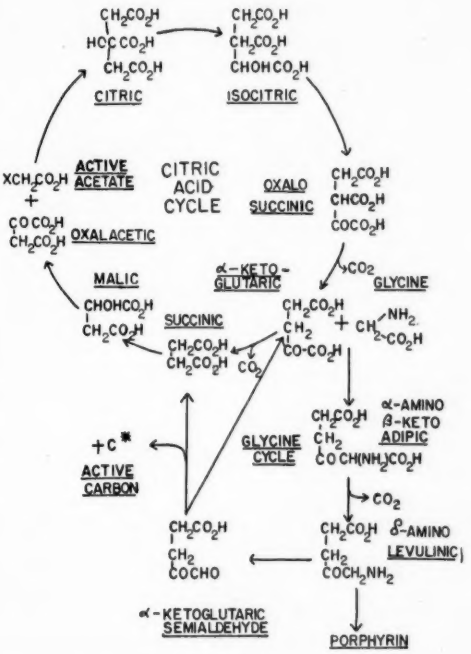

FIGURE V

The biosynthesis of porphyrin precursors by the tricarboxylic (citric) acid cycle (Krebs) and the glycine cycle (Shemin and Russell, 1953)

protein metabolism. Two molecules of this acid or a related succinyl compound may be condensed with glycine to yield a monopyrrole with acetic and propionic acid side chains in the β-positions. This theory, first read as a paper to the 1946 Australian and New Zealand Association for the Advancement of Science Congress, was published three years later (Lemberg and Legge, 1949). It was confirmed and proved by extensive studies of American workers, particularly the Columbia University group, and of English workers, Neuberger, Rimington and Falk (who had begun his studies in the porphyrin field in my laboratory) (Shemin and Wittenberg, 1951; Shemin and Kumin, 1952; Neuberger, Muir and Gray, 1950; Falk, Dresel and Rimington, 1953; Lemberg, 1954).

Alpha-ketoglutarate as well as succinyl-coenzyme A condenses with glycine to form δ-aminolævulinic acid, which Shemin and Russell (1953) have recently shown to be a precursor of hæm (Figure V). Two molecules of this acid are then condensed to form porphobilinogen by hæmolysates of birds' red cells (Figure VI), and porphobilinogen in turn yields not only uroporphyrin, but also coproporphyrin and the protoporphyrin of hæmoglobin in these preparations (Dresel and Falk, 1953; Falk, Dresel and Rimington, 1953). Porphobilinogen

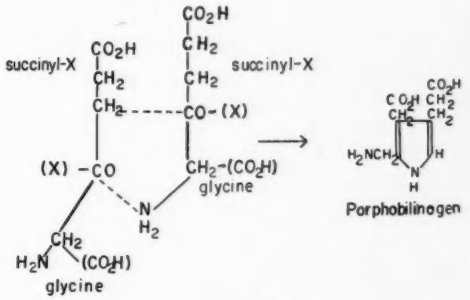

FIGURE VI

The biosynthesis of porphobilinogen from two molecules of δ-aminolævulinic acid. The latter is formed from glycine and α-ketoglutarate (X=CO_2H) or succinyl-coenzyme A. (X=—S radical of coenzyme A), with removal of the X groups, and the carboxyl groups of glycine

had been found by Waldenström and Vahlquist (1939) as a colourless precursor of uroporphyrin in the urine of patients with acute porphyria and believed to be a dipyrrole. It had been isolated in pure form by Westall (1952) in Rimington's laboratory, and shown to possess the monopyrrole structure given above. It has the structure of a monopyrrole with acetic and propionic acid side chains in β and a mono-C substituent in α postulated in my 1949 theory (Figure VII). While Watson (1950, 1951) believed he had evidence for a direct conversion of coproporphyrin to protoporphyrin, it now appears that the decarboxylations occur before final condensation of the porphin ring, probably in the monopyrrole stage for the conversion of uro-precursor to copro-precursor, and perhaps at a dipyrrolic stage for the oxidative decarboxylation of coproporphyrin to protoporphyrin as it was assumed in my theory (Lemberg, 1954). Preformed copro-porphyrin at least is not converted to hæm,

while the findings about uroporphyrin are controversial (Salomon, Richmond and Altman, 1952; Falk, Dresel and Rimington, 1953; Schwartz, 1954).

The synthesis of chlorophylls in green plants (Della Rosa, Altman and Salomon, 1953) and of bacteriochlorophyll in purple bacteria is according to the same pattern and also that of hæmatin enzymes in yeast and other micro-organisms and in leguminous root nodules.

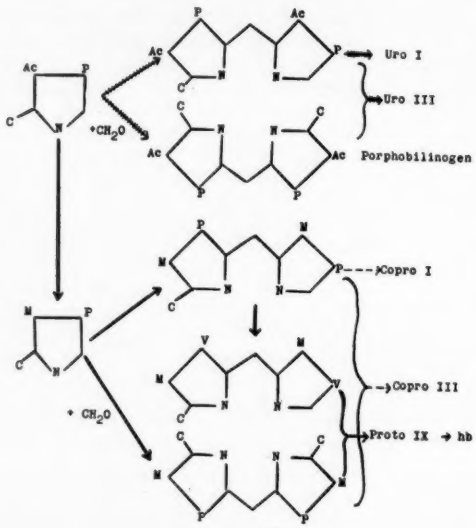

FIGURE VII

Porphyrin synthesis from monopyrrolic precursors (according to Lemberg and Legge, 1949). Major lines of synthesis are shown by straight lines capped by an arrow; minor lines of synthesis are shown by interrupted lines capped by an arrow; predominantly pathological lines are shown by lines crossed at intervals and capped by an arrow

PORPHYRINS IN DISEASE

A clear distinction must be made between porphyrinurias and porphyrias. The former is a symptom of many diseases and is characterized mainly by a moderate increase up to a few milligrammes per day of coproporphyrin. In contrast, the porphyrias are characteristic diseases of familial character, with uroporphyrin excretion increased to up to several hundreds of milligrammes.

Porphyrias. The symptoms of two of the porphyrias, chronic congenital porphyria and intermittent acute porphyria, are very different, but the third, *porphyria cutanea tarda*, has symptoms of both, and is by some considered as an intermediate type, although at least in some cases it has specific features.

Chronic Congenital Porphyria.—Chronic congenital porphyria is a very rare disease, and to my knowledge no case has yet been found in Australia. The genic aberration is recessive, and the disease is more frequent in males than in females. Its main symptom is due to photosensitization of the skin with severe scarring, which begins early in life. In some cases at least it is accompanied by hæmolytic anæmia and splenomegaly. A similar disease has been observed in cattle and pigs. The abdominal, neuromuscular and psychotic symptoms of intermittent acute porphyria are absent.

Large amounts of uroporphyrin and coproporphyrin are excreted and found widespread in the body, particularly in the bone marrow. The hæm synthesis in the bone marrow is disturbed; hence Watson calls the disease "*porphyria erythropoietica*" Uroporphyrin is deposited in the bones. The uroporphyrin of the urine is predominantly of type I. Porphobilinogen is absent. Schwartz, Keprios and Schmid (1952) have recently produced similar chemical symptoms in rabbits by a combination of lead administration, ultra-violet light irradiation and phenylhydrazine. It remains to be seen how closely this condition approaches congenital chronic porphyria.

Intermittent Acute Porphyria.—Intermittent acute porphyria is by no means as rare as chronic congenital porphyria. Genetically it is of dominant character with small penetrance. Some people appear to go through life without any symptoms of disease, although the chemical symptoms are present. In others, sudden attacks of all degrees of severity occur which may lead to death, or to equally sudden remissions. The disease appears most frequently at the twenty to twenty-five years age level, and is said to be more frequent in females.

The variety of symptoms is baffling, though skin photosensitization is conspicuously absent. The abdominal attacks of colic, vomiting and constipation are often so acute as to lead to unnecessary laparotomy. There is evidence for intestinal spasms with gross dilatation of colon and jejunum. Attacks are often preceded by loss of weight.

Attacks with peripheral neuropathy and psychotic disturbances are particularly serious, and may lead to death by paralysis of respiratory muscles. There are weakness, pain in the extremities or back, peripheral neuritis and paralysis. Microscopic examination reveals patchy degeneration of the myelin sheaths of

peripheral nerves, and chromatolysis of nerve cells of both horns and posterior root ganglia.

The later stages are often accompanied by severe psychoses and alteration of personality. Even these have been observed to recede in remission.

There is evidence for vascular spasms, and spasms of renal arteries may explain the kidney damage, the moderately increased blood urea content, the oliguria, and the hypertension, though hypotension has also been found. Sodium and chloride levels are low, perhaps owing to adrenal damage. There is also evidence for liver damage in attacks—increase of serum bilirubin and alkaline serum phosphatase levels, transient jaundice and ischæmic focal necrosis in the liver.

The most characteristic chemical symptom is the excretion of the colourless porphobilinogen which we have discussed above. It gives a red dye with Ehrlich aldehyde similar to that produced with urobilinogen, but differing from it by its insolubility in organic solvents (Watson-Schwartz test, 1941). Finding of porphobilinogen in the urine is almost pathognomic; but it is not always present in remission, occasionally not even in attacks. Heating of porphobilinogen in weakly acid solution leads to the formation of uroporphyrin III, while Lockwood (1953*b*) found that even in the absence of air and in alkaline solution porphobilinogen is slowly transformed into uroporphyrinogen, which has also been found to accompany porphobilinogen (Hawkinson and Watson, 1952). Uroporphyrin III must therefore constitute a substantial part of the uroporphyrin excreted in this disease. Waldenström, Fink and Hoerburger (1935) isolated from acute porphyria urine an ester of lower melting point than uro I ester, which he assumed to be uro III ester. According to Nicholas and Rimington (1953), this is substantially correct; but Watson, Schwartz and Hawkinson (1945) and Prunty (1946) found this ester composed of uro I ester with an admixture of a type III heptacarboxylic porphyrin ester. Perhaps the divergence is due to Watson and Prunty studying preformed uroporphyrin, Rimington the ester predominantly formed from porphobilinogen. Zinc, and more rarely, copper porphyrin have also been observed in acute porphyria.

According to Watson (Watson, Lowry, Schmid, Hawkinson and Schwartz, 1951), the seat of the abnormality is not the bone marrow, but the liver, and he thus considers this disease as one type of "*porphyria hepatica*". Uroporphyrin was found increased in the liver.

Recently, Schmid and Schwartz (1952*a*) and Goldberg (1954) have produced chemically similar symptoms in rabbits by "Sedormid" (allylisopropylacetylurea), allylisopropyl-acetamide, and allyl barbiturates. They also state that they have observed symptoms resembling those of acute porphyria, such as dilatation of the stomach, irregular bowel spasms and paresis of limbs. Of particular interest is the observation (Schmid and Schwartz, 1952*b*) that liver catalase was greatly decreased, although this has not been found in acute porphyria by Gray (1950). It may mean that the disturbance is one of hæmatin enzyme synthesis in the liver, not of hæmoglobin synthesis, and there is indeed no evidence of disturbed hæmatopoiesis in acute porphyria.

Porphyria Cutanea Tarda.—The third type of porphyria is a less clear-cut entity. It resembles chronic congenital porphyria in photosensitization of the skin; but this appears later in life and is less severe than in chronic congenital porphyria. It has therefore been named *porphyria cutanea tarda*. Its familial character has not yet been studied in detail. The skin of exposed parts has a violaceous hue, and bullæ can occasionally be caused by trauma and heat as well as by light. Abdominal, neuromuscular or psychiatric symptoms are rarer, but have been observed in attacks. The disease does not appear to be very rare, no less than 38 cases having been found in one large American hospital in a few years (Brunsting, Mason and Aldrich, 1951). There is evidence that the attacks are precipitated by liver damage, and can be caused by alcohol, chronic amœbiasis and hepatotoxic drugs. Macgregor, Nicholas and Rimington (1952) found in their case progressive arthritic changes and evidence of adreno-cortical deficiency, with gynic changes in a male.

However, the chemical symptoms appear to differentiate the disease more clearly from both chronic congenital and acute intermittent porphyria. Rimington found in remission very large amounts of coproporphyrin and protoporphyrin in the fæces (Table I). During an attack these decrease and are replaced by large amounts of uroporphyrin and coproporphyrin in the urine. It is well known that the less hydrophilic coproporphyrins (and protoporphyrin) are normally excreted by the liver into the bile and intestine, while the more hydrophilic uroporphyrin is excreted by the kidney. Liver damage decreases the ability of the liver to excrete coproporphyrin, and this leads to its increased excretion in the urine. Thus coproporphyrinuria is increased in liver diseases

TABLE I

Porphyrin Excretion in Milligrammes per Day (Approximate)[1]

Condition	Urine		Fæces		
	Uroporphyrin	Coproporphyrin	Uroporphyrin	Coproporphyrin	Protoporphyrin
Human being, normal	0·02	0·1–0·25	0	0·5	0·6
Lead poisoning	—	3	—	—	—
Congenital porphyria :					
Attack	>50	3	—	—	—
Remission	3	4	—	—	—
Acute porphyria :					
Attack	50	2	2	3	—
Remission	0·04	0·01	—	—	—
Porphyria cutanea tarda :					
Attack	50	10	0·5	—	—
Remission	10	1	—	10	50

[1] After Falk, 1954.

and jaundice. However, liver function impairment alone cannot explain the large uroporphyrin excretion in the urine in *porphyria cutanea tarda*, or the disappearance of free protoporphyrin from the fæces in an attack. The high protoporphyrin content of the fæces in remission appears to be a characteristic feature and has been found in relatives of a patient. Porphobilinogen is found only occasionally in this disease. The type of uroporphyrin excreted appears to vary.

Minor Disturbances of Porphyrin Metabolism.—Before an attempt is made to correlate the symptoms of porphyrias with the chemical findings, a short account of the less pronounced disturbances of porphyrin metabolism is necessary. Fæcal excretion has so far been little used, because of the complications introduced by bacterial synthesis of porphyrins in the intestine, and by bacterial conversion of hæmatin compounds in the diet into porphyrins (protoporphyrin and other dicarboxylic porphyrins). According to Larson and Watson (1949) there is no evidence for reabsorption of these porphyrins from the intestine. At present mainly two ways are open, the study of the free porphyrin content of erythrocytes and that of urinary excretion.

The former of these methods has the disadvantage that the amounts of free porphyrins in erythrocytes are small and may depend on the state of maturity of the red cell. There is for example, good reason to believe that the increases of erythrocyte protoporphyrin observed after the administration of vitamin B_{12} or folic acid by French workers (Bénard, Gajdos and Gajdos-Török, 1951) are due to this factor, rather than to increased porphyrin synthesis.

Finally red cells may not retain their porphyrin; for example, red cells in the bone marrow have been observed to contain more porphyrin than circulating cells. On the other hand, the amount of urinary excretion depends a good deal on the ability of the liver to excrete coproporphyrin into the bile.

Erythrocyte protoporphyrin, normally up to about 50 microgrammes per 100 millilitres of cells, is almost doubled in hæmolytic and refractory anæmias and in diseases of the leucocyte and lymphocyte systems (Ward and Mason, 1950). It is low in pernicious anæmia in relapse; but particularly high values, above 100 microgrammes per 100 millilitres of cells, are found in iron deficiency and lead poisoning. The increase is evidently due to a minor inhibition of iron incorporation, since there is a good deal of isotope and other evidence that erythrocyte-free protoporphyrin is formed simultaneously with the protoporphyrin in hæmoglobin (Gajdos-Török, Bénard, Coursaget and Gajdos, 1952).

Erythrocyte coproporphyrin content is usually below two microgrammes per 100 millilitres of cells (Watson, 1950). It is high in hæmolytic anæmia, but not in iron deficiency. It follows the reticulocyte count—for example, in pernicious anæmia treatment—far more closely than does erythrocyte protoporphyrin content. Traces of uroporphyrin are also present.

The increase of coproporphyrin in the urine is found in liver disease, for the reason mentioned above, but also in a variety of other diseases. It can be said in general that this increase is predominantly due to coproporphyrin I in diseases in which an increased hæmatopoiesis counterbalances increased destruction, while in aplastic anæmias and derangement of hæmato-

poiesis by drugs and heavy metal, notably lead, coproporphyrin III prevails. Alcohol belongs to this latter class; while coproporphyrin I predominates in other liver diseases, type III is found in alcoholic cirrhosis. However, in detail, the matter is still far from clear. For example, coproporphyrin III is also increased in poliomyelitis; but this does not appear to be derived from the very small amounts found in the nervous system. Coproporphyrin I is said to prevail in leuchæmia, but coproporphyrin III in Hodgkin's disease. Coproporphyrin III has also been found in elliptocytosis. In yeast maximal coproporphyrin formation is accompanied by maximal III : I ratio, and coproporphyrin III is particularly high in carbohydrate starvation. The free porphyrin formation in yeast is probably connected with a disturbance of cytochrome formation—not as Kench (1954) now believes, on rather scanty evidence, from cytochrome decomposition.

There is increasing evidence that in lead poisoning, as well as in acute porphyria, coproporphyrin is excreted as a colourless precursor. This does not appear to be coproporphyrinogen, since it is not converted to porphyrin by iodine, but is so by acid. It has been assumed by some to be a metal complex, but Latsen and Orten (1954) removed coproporphyrin, and presumably also its metal complex, from acute porphyria urine by adsorption to talc, leaving a precursor convertible to coproporphyrin by acid. This may well be a coproporphobilinogen—that is, the monopyrrolic precursor with methyl and propionic acid side chains postulated in my 1949 theory. The condensation of this with (uro)porphobilinogen would yield the pentacarboxylic, hexacarboxylic and heptacarboxylic acids.

Weatherall (1952) found that coproporphyrin III injected into lead-poisoned rabbits, was excreted mainly in the bile, not in the urine; so that it is not liver impairment, but excretion as precursor, which determines the urinary excretion in lead poisoning. A monopyrrolic precursor would indeed be more hydrophilic and therefore more likely to be excreted in the urine. The disturbance in lead poisoning is multiple. Small amounts of uroporphyrin and much coproporphyrin are excreted, while in the erythrocytes protoporphyrin is increased. The first decarboxylation (uro to copro) is thus only slightly interfered with, in contrast to the porphyrias, but both the secondary decarboxylation (copro to proto) and the iron incorporation into protoporphyrin are affected.

Ætiology of Porphyrias.—Any attempt to correlate the chemical aberrations of porphyrin metabolism with the symptoms of porphyrias is still faced with insuperable difficulties. The photosensitization of the skin in chronic congenital porphyria and in *porphyria cutanea tarda* is obviously due to the porphyrin, particularly uroporphyrin I, and the lack of this symptom in acute intermittent porphyria is perhaps due to its excretion in the form of a precursor in this disease. Many of the symptoms found in intermittent acute porphyria have, under certain conditions and at one time or another, been produced by porphyrins—for example, vascular and abdominal spasms and liver damage. Kidney damage in turn may be due to vascular spasms. But in such experiments the porphyrin used was usually the unphysiological hæmatoporphyrin, and recent experiments with the physiological porphyrins have shown them to be pharmacologically almost inactive (Goldberg, Paton and Thompson, 1954). It also remains a mystery why these symptoms are not found in chronic congenital porphyria, in which more preformed uroporphyrin is present. Nor are severity of symptoms and porphyrin excretion parallel in many instances of acute porphyria. Porphobilinogen does not cause these symptoms, and one may have to go back further to abnormal products in the stages preceding porphobilinogen synthesis.

Nor is it clear what is the cause of the neural damage in acute porphyria. Since demyelination has been found in inhibition of cytochrome oxidase by cyanide (Wyndham, 1941), one may speculate on a derangement of cytochrome metabolism combined with porphyrin formation, parallel to experiences with yeast. The experiments of Granick and Gilder (1945) with *H. influenzæ* indicate that porphyrins different from protoporphyrin may inhibit the combination of the latter with protein to form hæmatin enzymes. Klüver (1944) has observed that coproporphyrin occurs in white matter, particularly in regions devoid of cytochromes. He has recently shown (Klüver and Barrera, 1954) that copper phthalocyanine, somewhat related to porphyrins, is preferentially adsorbed by the myelin sheath; but he has not found any evidence for accumulation of porphyrin in the central nervous system in porphyria.

It is also still difficult to account satisfactorily for the different chemical findings. The 1952 scheme of Rimington can already be shown to be wrong in some details. The assumed enzymic control of uroporphyrin type III synthesis as compared with that of type I is

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apparently contradicted by the in-vitro conversion of porphobilinogen into uroporphyrin III, nor does the assumed direct conversion of one porphyrin into another appear to be correct. In the porphyrias the main feature appears to be inhibition or failure of enzymes decarboxylating acetic acid side chains of porphobilinogen to methyl, combined with a minor disturbance of the enzymes decarboxylating the propionic acid groups to vinyl. Only in chronic congenital porphyria do these disturbances affect hæmatopoiesis, and this by producing unstable red cells rather than by decreasing protoporphyrin and hæm synthesis; hæmatopoiesis is increased to counterbalance the hæmolysis, and this contributes to the increased formation of type I uroporphyrin.

Finally, the chemical mechanism of synthesis of type I and type III porphyrins is still not understood. Type III synthesis is possible only if a monocarbon compound combines two monopyrroles symmetrically to a dipyrrole, and such a dipyrrole then condenses preferentially with an unsymmetrical dipyrrole produced by autocendensation of the monopyrrole. Several schemes for such a synthesis depending partly on relative reaction rates have been devised, but none is free from specious assumptions. The monocarbon compound may be assumed to come either from porphobilinogen itself (for example, from the aminomethyl group), or from the original Shemin cycle—that is, from reconversion of δ-aminolævulinic acid to succinyl-coenzyme A. It is in reactions of this type that one may expect to find pantothenic acid (a constituent of coenzyme A), pyridoxine (a catalyst of transamination), or folic acid (a monocarbon catalyst) to be active. Some of the symptoms of acute porphyria are perhaps caused by unknown metabolites produced by aberrations in δ-aminolævulinic acid metabolism.

CONCLUSION

Much work still needs to be done in this field. We can expect further important help from tracer methods, and these must be also applied to the study of the synthesis of the hæmatin enzymes. The methods of isolation and separation of porphyrins need further refinement, and more attention must be given to the colourless precursors. Pharmacological experiments must be carried out with the natural porphyrins, rather than with hæmatoporphyrin. Finally, the field is now open for exploration of the enzymes which catalyse the processes, and about which we know nothing so far.

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A MODEL REPRESENTING THE CONTROL OF BODY FLUID VOLUME IN MAN[1]

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In a number of papers over the past five years certain salient observations on the control of fluid volume in man have been recorded (Lowe, 1950, 1951, 1952, 1953; Lowe and Sayers, 1952). It has been suggested on the basis of these observations that the total fluids of the body represent an open system, into which a continuous flow of fluid takes place and from which a continuous outflow occurs, and that the amount of fluid stored in the body at any instant influences both the intake and the output of fluid. Further, this hypothetical system must be complex and contain at least two components. The observations made indicate that unless a circulation is incorporated in this hypothetical system, some of the clinical observations cannot be explained.

On the basis of these observations, and of other facts already established in physiology, a model has been constructed in which two open systems, each containing pump-driven circulations, are linked with each other. This model has enabled the implications of the hypothesis to be checked against the naturally occurring phenomena of many diseases which are associated with disturbance of body fluid regulation. On a qualitative basis a very good correlation has been obtained between the model's behaviour and clinical observations in a large number of conditions.

Observations

The pertinent observations upon which the hypothesis is to be made may be summarized under the headings of volume regulation, fluid intake, fluid output, body fluid compartments, circulation and receptor mechanisms.

Volume Regulation

In a normal adult regulation of total fluid volume is not absolute, for the volume oscillates between close limits which may be held over long periods. Our observations on fluid balance (Lowe, 1953) indicate that this range is at least of the order of ±500 millilitres (Figure I), a finding which is in accord with the studies of Dodds (1950) on the maintenance of body weight.

When a person develops generalized œdema, the balance of the system is disturbed and the fluid volume increases, at first slowly, then rapidly, and finally slowly (Lowe, 1953). In recovery from œdema the fluid volume diminishes in a similar fashion, and then fluctuates until its oscillations stay within the normal limits for that individual (Lowe, 1951; Figure II).

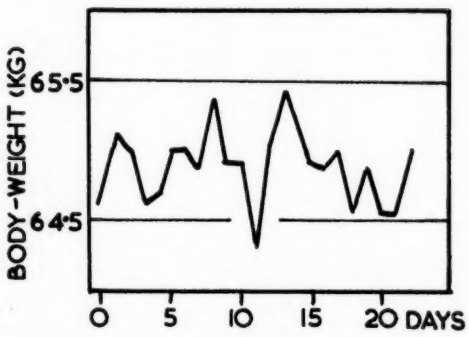

Figure I

The daily weight of a normal person, which has been recorded under standard conditions and considered to represent the variation in body fluid volume

Fluid Intake

The fluid which enters the body comes from the fluid drunk, the fluid of solid food and the water of metabolism produced within the body itself. In addition to water much of this fluid also contains electrolytes; but electrolytes, chiefly sodium chloride, may also be taken in the food in solid form.

However, there is the evidence of common experience that thirst may be for water alone

[1] Received on September 10, 1954. Part of the expenses of this investigation were defrayed by a grant from the National Health and Medical Research Council.

or for drinks containing salt. Water thirst and salt hunger are separate desires. This implies that the intake of water and that of electrolytes may be controlled separately, even though the two are usually supplied concomitantly.

Experimental observations on man and on animals show that both volume and osmotic pressure changes in body fluids influence thirst. Gilman (1937) showed that cellular over-hydration reduced the fluid intake of dogs, and Adolph that pure water volume deficits in man, dog and burro were exactly corrected by water drinking (Adolph, 1939, 1950; Barker, Adolph and Keller, 1953).

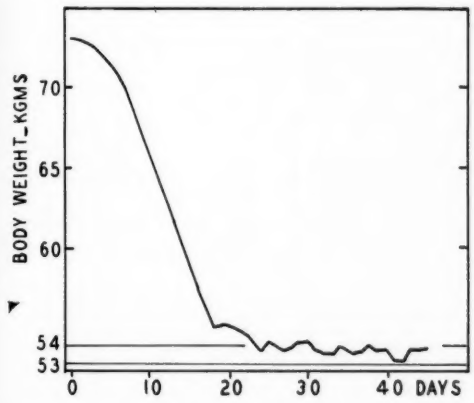

FIGURE II

The daily weight of a patient recovering from cardiac œdema. This is considered to represent the variation in body fluid volume. Note that over the last fifteen days the weight fluctuates within ±0·5 kilogramme of the patient's mean normal weight

The latter authors also showed in the dog that excessive intake of salt produced prompt drinking of water. Gilman (1937) was able to show that elevation of blood osmotic pressure led to a water thirst such as to produce exact restoration of the normal osmotic pressure. Holmes and Gregersen (1947) observed the effects on plasma volume and blood electrolyte concentration of intravenous infusions of hypertonic saline. From their results it would seem that, in some instances at least, osmotic pressure elevation is dominant over water volume increase in the stimulation of thirst. However, the stimulus for salt hunger is unproven. In one of our patients salt hunger was present whilst his serum sodium and chloride levels were respectively eight and 14 milliequivalents per litre below his normal values.

Our studies on congestive cardiac failure show that changes in fluid volume of the body influence patients' intake of water, and sometimes of salt.

Fluid Output

Fluid lost through the lung and skin probably represents a temperature regulating mechanism and plays little part in the normal regulation of body fluid volume (Peters, 1952). From the point of view of fluid volume regulation, fluid is lost from the body through the kidney, and this loss is influenced both by the volume of body fluids and by their osmotic pressure.

In 1951 Welt and Orloff noted that expansion of plasma volume by iso-oncotic albumin solutions increased the rate of water excretion through the kidneys. The association of changes in blood osmotic pressure with renal water excretion was shown by Verney (1946).

Seldin and Tarail (1949) have shown that change in both volume and osmotic pressure of body fluids influences the excretion of electrolytes by some central action, and not by their local influence in the renal tubule during excretion.

Our observations, in particular with œdema, show that frequently the intake and output of water (Lowe, 1953) and of electrolytes are influenced in a reciprocal manner by changes in volume.

Sawyer (1952) has discussed the physiological antagonism between posterior pituitary and adrenal cortical hormones in the maintenance of homœostasis; these hormones are concerned in the control of the renal excretion of the electrolyte solution.

Body Fluid Compartments

The body fluids are distributed chiefly in three compartments—the intravascular, the extracellular extravascular, and the intracellular—which are separated by semi-permeable membranes. Although local and general agents may alter the partitioning of body fluids in these compartments, they do not alter the overall behaviour of the system. The changes in intake, outflow and volume are qualitatively the same in recovery from excess fluid volume, whether the excess is in one, two or all of the compartments; for example, the same pattern of recovery is seen in ascites from liver disease, in œdema from nephritis, in cardiac failure and in nephrosis.

Circulation

Connecting the compartments of the body fluid with the intake and output mechanisms is the blood vascular system. That disturbances

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in this system can produce changes similar to those already commented upon is well illustrated by a study reported from this centre (Lowe, 1953) concerning a patient with a hæmopericardium.

Receptor Mechanisms

There is considerable evidence that there exist in man receptors which are influenced by change in volume of some part of the body fluid, and others which are sensitive to changes in osmotic pressure of some part of the body fluids. Verney (1946) has produced evidence for the existence of an osmoreceptor situated in the field of supply of the common carotid artery, probably of the internal carotid artery, which influences the secretion of posterior pituitary antidiuretic hormone, and so controls water outflow. Welt and Orloff (1951) record results which indicate a receptor sensitive to change of plasma volume which influences water outflow. Nelson *et alii* (1954) think this to be situated in the supra-optico-hypophyseal system. Andersson (1953) states that nerve cells in the region of the hypothalamus and the third ventricle are part of a pathway linking thirst with changes in osmotic pressure of body fluids. However, Gauer *et alii* (1951) locate in the great veins of the thorax a receptor which influences urine flow. Leaf and Couter (1949) and Alexander *et alii* (1951) have produced evidence which indicates that the anterior pituitary (ACTH) and adrenal cortical hormones form an effector agent of this control in so far as sodium excretion is concerned.

Viar *et alii* (1951) showed that compression of neck veins in man leads to an increase in sodium outflow from the body. Luske and Palmer (1953) likewise produce evidence for a "quantometer" separate from Verney's "osmometer", which is actuated by volume change and influences sodium outflow.

It appears likely, therefore, that in man there are at least two receptor mechanisms in the body fluid control system, and that these are sensitive to change in volume and osmotic pressure. They influence the outflow of water and electrolytes and possibly also the inflow of both.

Hypothesis

From these observations, and bearing in mind that the fluid contains both solvent and solute we may construct the following hypothesis: the fluid storage within the body and the flow of fluid through the body represent an "open" storage system, in which the fluid in the reservoir actuates controls over the inflow and outflow of fluid. This concept may be represented diagrammatically as in Figures III and IV.

In such a system, if we consider it to contain only one component, say, water—three general features can be readily appreciated. First,

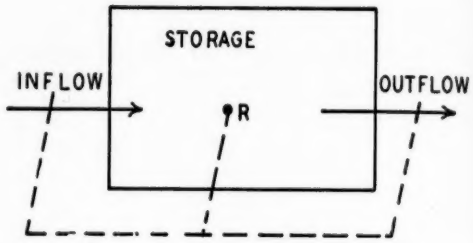

Figure III

Diagrammatic representation of an "open" system with continuous inflow and outflow controlled by a receptor (R) within the system. Interrupted line indicates linkage between receptor and inflow and outflow controls

there exists an equilibrium point which determines the volume of the storage, if there is a state of the reservoir at which outflow and inflow are equal. Secondly, oscillatory changes

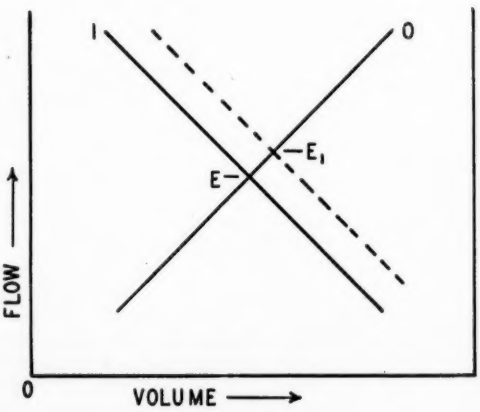

Figure IV

Chart to show the changes induced in inflow (I) and outflow (O) by changes in volume in the system. An equilibrium point (E) is present where the lines intersect and inflow equals outflow. The broken line indicates a different volume-inflow relationship giving a different equilibrium point E_1

may occur in the behaviour of its component parts. Thirdly, any receptor mechanism in the reservoir need be sensitive only to change of volume in the storage, and need not be set to an absolute value.

If the inflow and outflow in such a system are influenced in a reciprocal manner by change in storage content, then, as is seen in Figure IV, there will be an equilibrium point (E) at which inflow is equal to outflow, and the system will be maintained at this point. In the figure the relation of inflow and outflow to volume is shown as linear, but this is probably not true *in vivo*. It is more likely that the lines are curved; however, so long as the curves intersect there will be an equilibrium point.

If this system is disturbed from equilibrium, its inertia will cause it to oscillate about the equilibrium point as it returns to that position. Further, the steeper the curves at the point of intersection, the more stable will be the system, and the smaller the range of oscillation about the equilibrium point. Conversely, the more gradual the slope of the intersecting curves, the greater the range of oscillation about the equilibrium point.

Provided the receptor mechanism which links the storage with the inflow and outflow mechanism is sensitive to change in storage, there is no need for it to be sensitive to any absolute value of the contents. The actual equilibrium point will be determined by the characteristics of the reservoir, of the inflow and outflow mechanism, and of the control links. If any of these characteristics change, then the equilibrium point is also likely to change—as, for example (Figure IV), a change in the volume-inflow relationships will set a new equilibrium point (E_1) at a new volume.

In view of the complex nature of body fluid, it is to be expected that the body fluid system will contain more than one such "open" system, and that these several systems may be interlinked. These complex "open" systems will also show the three features already described—namely, equilibrium points for the controlled components, oscillatory behaviour, and the need for the receptors to be sensitive only to change in some value of the various components.

Our previous studies on models with hydraulic "open" systems (Lowe, 1953) have suggested that many of the phenomena of fluid balance seen in clinical states could be accounted for by a combination of two such systems. However, these models lacked both multiple fluid compartments and a circulating mechanism, and so were limited in their analysis of the problem. Further, they did not employ an outflow valve designed on the principles of the mammalian kidney.

Inspired by the model circulation constructed by Starr and Rawson (1940), and the partial success of the previous simple models, an hydraulic system containing a circulation, compartmented fluid reservoirs, two "open" systems and a complex outflow valve was constructed.

As body fluid is a solution of electrolytes and there is evidence that the solvent and solute are handled to a large extent separately, it was decided that one system should represent water and the other electrolytes. Although the electrolytes of the body fluids are numerous, for simplicity they are treated as a simple substance. The mechanical components of these two systems were as far as possible made identical.

Basically each system contains an inflow valve, from which liquid passes into the low pressure side of a pump-driven circulation. From the circulation opens a reservoir, which is compartmented. To the high pressure side of the circulation an outflow valve is connected. This complex valve contains a mechanism to feed back a portion of the outflow into the low pressure side of the circulation.

Operation of the valves is controlled directly or indirectly by the volume of liquid in various parts of the system.

THE MODEL

Figure V is a diagrammatic representation of the model incorporating the basic ideas just described.

Inflow

The inflowing liquids come from a constant level reservoir and pass through regulating valves. In the case of the "water" (IN w) side, the liquid passes through two valves in parallel—one (V_3) actuated by the volume in the main "water" reservoir (W), and the other (V_2) by the ratio of the height in the "water" reservoir and the "electrolyte" reservoir (E). This ratio is taken to represent osmotic pressure for the following reason: electrolyte solutions in the body are mainly fully ionized, so that osmotic pressure can be represented by the ionic concentration which is a ratio of mass to volume. In this system W represents volume and E the mass of electrolytes, so that ionic concentration can be represented as the ratio of W to E. These parallel valves therefore represent "water" and "osmotic pressure" control of the intake of "water".

The liquid representing "electrolyte" inflow (INE) passes through only one regulating valve (V_1). As there is no evidence available as to the normal stimulus of salt hunger, this "electrolyte" inflow valve has been controlled by "osmotic pressure". Of the possible

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sources of control, this has been found to give the most satisfactory correlation with the clinical findings.

After passing the inflow valves the " water " inflow goes through a recording flow-meter (IR w), and then by hydrostatic pressure into the low pressure side of the " water " circulation.

The " electrolyte " inflow passes directly into the low pressure side of the " electrolyte " circulation, and its amount can be recorded directly from the position of its inflow valve.

representing together the fluid of the circulating intravascular fluids. In each of these reservoirs are floats (F1, F2).

Opening from each reservoir is a second one (W′, E′). The primary reservoirs (W, E) are connected with secondary ones (W′, E′) by a tube of variable diameter, which can represent the semi-permeable membranes between vascular and extravascular fluid compartments.

Attached to the primary " water " reservoir is a volume recorder (VR).

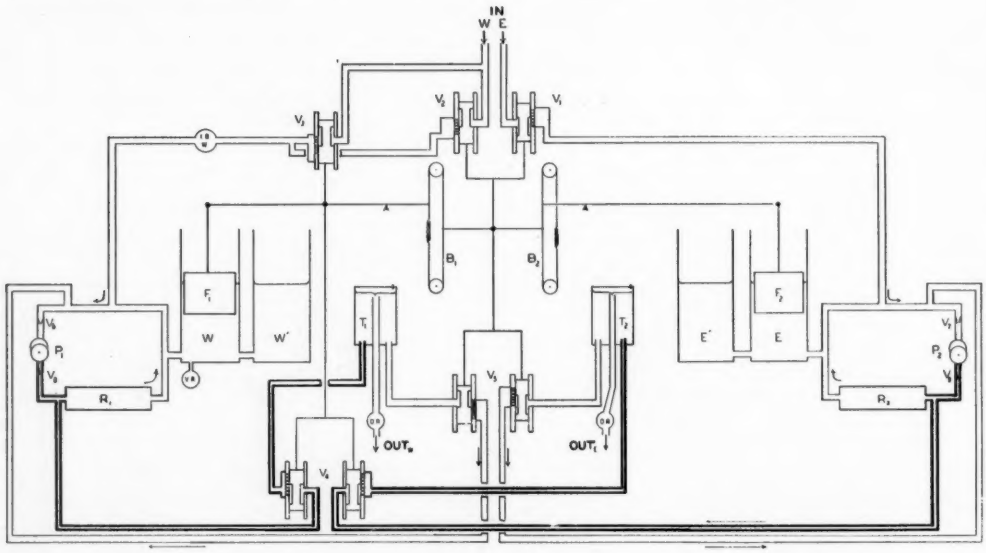

FIGURE V

Diagrammatic representation of the components of the model and their linkage. B_1B_2, endless, spring-loaded belts for " OP " measurements; EE′, " electrolyte " reservoirs; F_1F_2, floats actuating valves; INW, " water " inflow; INE, " electrolyte " inflow; IRW, " water " inflow recorder; OR, Outflow recorders; OUTW, " water " outflow; OUTE, " electrolyte " outflow; P_1P_2, circulation pumps; R_1R_2, hydraulic resistances in circulation; T_1T_2, outflow storage tanks; V_1, " electrolyte " inflow valve; V_2V_3, " water " inflow valves; V_4, outflow valves; V_5, opposed feed-back valves; V_6V_7, pump inlet valves; V_8V_9, pump outlet valves; VR, volume recorder; WW′, " water " reservoirs. ▲▲, fulcrums for linkage arms. Arrows indicate direction of flow. Solid circles on linkages indicate hinge joints. Heavy lines indicate high pressure regions. Single lines indicate low pressure regions

The Circulation

The circulations each consist of a circuit of rubber tubes containing a Dale-Schuster pump (P1, P2) associated with one-way bicuspid valves (V6, V7, V8, V9) and a hydraulic resistance (R1, R2). The rubber tubing is thin-walled and of wide bore in the low pressure parts, and thick-walled with a small bore in the high pressure portions.

Reservoirs

Connected to the low pressure parts of the circulations are the reservoirs (W and E)

Outflow

Leading from the high pressure part of the circulation is a tube to the outflow valve mechanism. This mechanism is based on the following principles of renal function: first, the vascular fluid (electrolyte in solution) is filtered from the circulation through the glomerulus; and secondly, a large proportion of this fluid is reabsorbed into the circulation by the tubule cells. There is considerable evidence that the reabsorption of the solvent (water) and that of the solute (electrolyte) of

this fluid are to a large extent under independent control.

The control of filtration is determined by the vasomotor mechanisms of the afferent and efferent arterioles of the glomeruli in particular, and in general by the vasomotor control of the pressure and flow pattern in blood vessels throughout the body. What is the afferent stimulus for this control is uncertain. This model contains no analogue of the vasomotor mechanism for controlling the distribution pattern of blood or of the pressure-flow pattern in the body. However, it was found most satisfactory to control the flow of both " water " and " electrolyte " through the valves (V4) from the " water " volume, and this accords with the observation of Wesson *et alii* (1948) that glomerular filtration is to some extent controlled by compartmental volume imbalance. These valves represent the glomeruli.

Tubular reabsorption is greatly influenced by the hormones of the posterior pituitary lobe (anti-diuretic hormone) and of the adrenal cortex, the first being mainly concerned with water reabsorption, the latter with electrolyte reabsorption. It is likely that these hormones are secreted in response to osmotic pressure changes (Verney, 1946). However, osmotic pressure changes influence these secretions in opposite directions.

In the model, after passing the " glomerular " valves (V4) the " water " and " electrolyte " pass into reservoirs (T1, T2) at a height sufficient to provide a hydrostatic force for feed-back. From these reservoirs some liquid passes to the feed-back valves (V5), which are so constructed that they are controlled in inverse directions by " osmotic " pressure. From these valves the liquid is returned to the low pressure side of their respective circulations. Liquid which is not fed back into the circulation escapes to the outside through the outflow flow-meters (OR).

Control Mechanisms

The control mechanisms postulated are actuated either by volume of some part of the " water " system or by " osmotic pressure ", and operate through valves of tubular design in which a moving piston controls the flow. The " water " volume receptor is represented in the model by the float (F1) and the levers directly connected to it. These are so arranged that a rise of volume in the reservoir (W) diminishes the " water " inflow through the valve V3 and increases the outflow of " water " and " electrolyte " through the valve V4. " Osmotic pressure " is represented in the model by the difference in height between the " water " and " electrolyte " reservoirs (W and E). This difference is measured by the lever systems starting in the floats (F1, F2), and linked through the endless belts (B1, B2) to a central vertical rod which operates valves (V1, V2, V5). If the height of F2 becomes greater than F1, then the " osmotic pressure " of the system may be considered to have increased. This change is arranged to increase the " water " inflow through the valve V2, to decrease the " electrolyte " inflow through the valve V1, to increase the feed-back of " water " and to reduce the feed-back of " electrolyte " in valve V5.

It is to be noted that, all the valves being of identical design, all controlling factors, whether on inflow or outflow, " water " volume or " osmotic pressure ", are given equal weight. This is certainly not so in the intact animal, but the relative importance of the controls is not known. In fact, some individuals seem to correct disturbances by changes in output, others by changes in intake, and others by both (Kanter, 1953 ; Barker *et alii*, 1953), so probably the relative importance of the controls varies from animal to animal and from time to time.

Recorders

In studying the behaviour of the model continuous records of many variables are necessary. These have been made by means of levers on a kymograph. The levers are connected to the various flow-meters and tambours by strings and pulleys. Similarly the position of pistons in valves or of various levers can be recorded by strings and pulleys to similar kymograph levers. Pressures in various parts of the circulation can also be recorded by floats on mercury manometers.

Behaviour of the Model

In this model adjustments can be made to a number of the controls, so that inflow and feed-back of " water " and " electrolyte " can be altered. Changes in volume and " osmotic pressure ", and of partition of fluid can be impressed on the system. Changes in pumping power and leaking circulation valves can be produced. These changes can be made as primary changes and the response of the system studied. Further, any combinations of these changes can be made.

As clinical observations (Lowe, 1953) have suggested that it is the early changes which show clearly the direction and movement of these variables, these have been particularly studied in the model. As the relative importance

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of the various controls is not known, no detailed analysis of the changes seen when more than one variable is altered has been presented. In Figure VI the direction of changes in " water " volume both vascular and extravascular, in " osmotic pressure " and in inflow and outflow of " water " and " electrolyte ", in response to various experimental conditions is recorded. The heavy arrows indicate the primary change impressed on the system.

Two typical recordings are shown in Figures VII and VIII.

feed-back of " water " and " electrolyte " correspond respectively with *diabetes insipidus* and Addison's disease.

Excessive " water " and " electrolyte " loading correspond to clinical states sometimes seen in patients treated parenterally, rather than to spontaneously occurring disease states.

The effects of loss of pumping power correspond to cardiac failure with œdema. If this loss is due to actual loss of pumping power *per se*, it corresponds to " forward " failure. If it is due to a leaking pump inlet valve, it

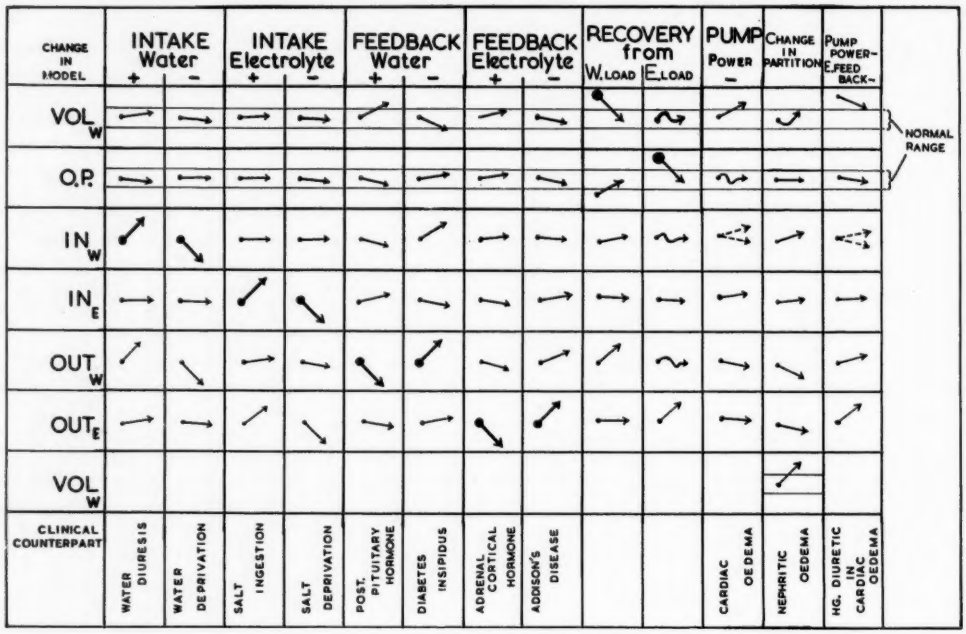

FIGURE VI

Correlation with Clinical Conditions

The situations represented in Figure VI are considered to correspond in many ways with known clinical states. The responses of the components recorded also agree with those seen clinically, in so far as they have been recorded in man or animals either by ourselves or by others (Wolf, 1950).

Forced increase of intake of " water " or " electrolyte ", and the forced decrease of intake of these, correspond to clinical conditions readily demonstrated in normal individuals.

Forced increases in feed-back of " water " and of " electrolyte " correspond respectively to the exhibition of posterior pituitary hormone, and adrenal cortical hormone. Decreased corresponds to " backward " failure. In both cases there is a change in the pressure pattern in the circulation, with diminished outflow through the " glomerular " valves. In the backward type of failure there is a conspicuous rise of pressure in the low pressure parts of the circulation, corresponding to venous congestion. This is not marked with straightout pumping power failure. In the model the direction of the responses is the same with either form of pump failure. The importance of local vasomotor control in the kidney has been shown by Miles *et alii* (1954) and by Pullman and McClure (1954), and the absence in the model of the local mechanism as well as the general one to control the distribution of fluid throughout the

system is probably responsible for unmasking the similarity in behaviour in both " forward " and " backward " types of heart failure.

Change in partition of liquid in the system can be brought about by emptying the reservoirs W′ and E′ and allowing them to fill slowly through a slightly open connecting tube. This is equivalent to clinical conditions in which there is a pre-renal deviation of water, such as occurs in œdema associated with low blood protein levels, or ascites from cirrhosis of the liver. It represents the formation of œdema from an imbalance between osmotic pressure and filtration forces in the capillaries.

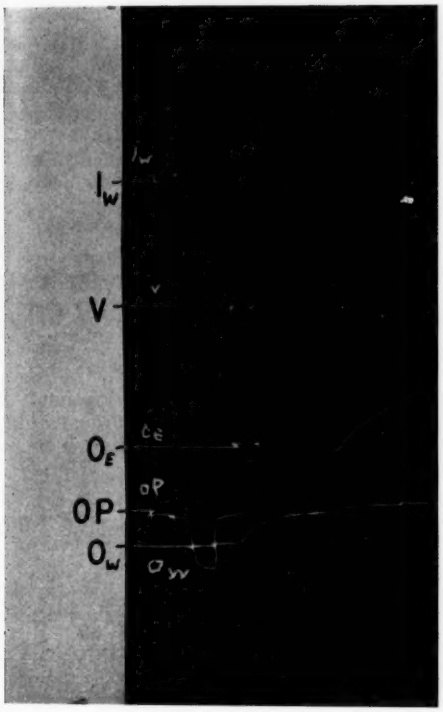

FIGURE VII

Photograph of kymograph record showing model response to a water load indicated by the spike on the volume curve (V). I_W, " water " inflow; V, volume of " water " reservoir; OP, " osmotic pressure " of system; O_E, outflow of " electrolyte "; O_W, outflow of " water ". Small vertical marks on tracings indicate simultaneous points. Note that inflow of " electrolyte " is indicated by the reciprocal of the OP record. In this instance there is a rapid correction of the system by the inflow mechanism and a slower correction by the outflow mechanism

The final condition illustrated in Figure VI is a combination of factors equivalent to the administration of a mercurial diuretic in congestive cardiac failure, the effect of the mercurial diuretic being to reduce the feed-back of electrolyte through the renal tubules. It is seen from Figure VI that in some respects the response of the system is variable, and depends on the relative importance of osmotic and volume control. A similar situation exists in cardiac failure, as has been shown in clinical observations (Lowe, 1953).

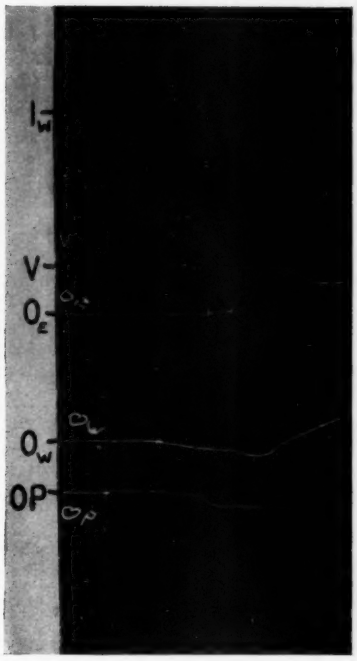

FIGURE VIII

Photograph of a kymograph recording showing model response to reduction of " electrolyte " feedback which resembles Addison's disease. I_W, " water " inflow; V, volume of " water " reservoir; OP, " osmotic pressure " of system; O_E, outflow of " electrolyte "; O_W, outflow of " water ". Note small change in " water " volume and " osmotic pressure ", small changes in inflow mechanism, but big changes in outflow mechanism

DISCUSSION

From clinical observations on the control of body fluid volume in man, an hypothesis concerning the mechanisms of control has been

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proposed. This hypothesis postulates a complex "open" system with a storage of fluid within the body, through which passes a continuous flow of fluid. The fluid is stored in various compartments separated by semi-permeable membranes. The inflow sites (alimentary canal and tissue cells), the various parts of the body and the outflow site (the kidney) are linked by the circulatory system. The body fluid is an electrolyte solution, both the solvent and solutes of which are to some extent independently controlled. The total volume and the electrolyte concentration of body fluid are normally kept within a narrow range by regulation of inflow and outflow of water and electrolytes. This regulation is actuated by changes in volume of some part of body fluid compartments and by changes in the osmotic pressure of some portion of the body fluid.

As this postulated mechanism, whilst simple in concept, is too complex to allow of easy mathematical analysis, a mechanical model has been constructed to study its implications. This model has been based on a complex open storage system, with a circulation and inflow and outflow mechanisms and controls suggested by physiological studies.

In constructing the model it was necessary to make assumptions concerning some controls, of the nature of which no physiological evidence is available. In particular the control of electrolyte inflow and the flow of water and electrolyte through the glomerular valves are unknown. After experiments with various linkages, it was found that the best correlation with clinical findings was obtained when the inflow of electrolytes was determined by the osmotic pressure of the system and the flow through the glomerular valves by the fluid volume. Possibly these are the sites of the afferent parts of these mechanisms in man.

In studying the behaviour of the model no attempt has been made to get quantitative results, because we are ignorant of the quantitative relationships of the various components. Thus no evidence is available to indicate the relationships between volume of fluid and osmotic pressure on the one hand, and the inflow and outflow of water or electrolyte on the other. From Figure IV the importance of these relationships is apparent, for any change in them, by disease for example, will alter the equilibrium point of the system and could lead to œdema or dehydration, to increase or decrease of osmotic pressure.

With the methods of investigation at present available it seems unlikely that these quantitative relationships can be determined. Whilst the change in storage of fluid in the body can be measured in many instances with an accuracy better than 0·5%, changes in the various aspects of renal function can rarely be measured with an error of less than 5%. The actual changes in outflow of fluid through the glomeruli or its reabsorption through tubules in patients becoming œdematous or recovering from œdema are frequently of the order of 1% or less, values well within the experimental error (Wesson *et alii*, 1948). Further, the osmoreceptor is sensitive to changes of 2% in osmotic pressure (Verney, 1946).

The qualitative agreement between the behaviour of the model and the observed behaviour of fluid storage and control in man, both normal and in the presence of disease, is so good that it suggests that the basic principles incorporated in the model are a close approximation to those occurring in man.

If this is so, then the hypothesis indicates gaps in our knowledge and lines for future investigation. These must be directed to a study of the quantitative relationships between inflow, outflow and storage of body fluid, and of the nature of the control of inflow and outflow by changes of both volume and concentration in the storage.

Present knowledge suggests that these linkages are complex and involve both nervous and hormonal components. The receptors for osmotic pressure have been shown to be in the brain, and those for volume probably also reside there. On the efferent side are the hormones which influence water and electrolyte outflow, and the nervous and hormonal mechanisms which influence the distribution of the circulation to various organs. Nothing seems to be known of the efferent pathways for thirst and salt hunger control.

It is of interest to note that the hypothetical system provides a basis for synthesis of the rival hypotheses of "forward" and "backward" cardiac failure, and shows a mechanism which includes the heart pump and circulation as an integral part of the fluid regulation. This provides a concept which overcomes the paradox noted by many writers (Starr, 1940) that the heart could be fitted only with difficulty into the scheme of things in the production of œdema in cardiac failure.

Summary

A study of the regulation of the volume of body fluids has been made with a mechanical model.

This model is based on the hypothesis that fluid storage within the body and flow of fluid

through the body represent an " open " storage system, in which the fluid in the reservoir actuates controls over the inflow and outflow of fluid. The model contains two " open " systems, which represent " water " and " electrolyte ", compartments which represent the body fluid compartments, a circulation and outflow valves designed on the principles of renal function.

With this model it has been possible to reproduce the water and electrolyte changes seen in the clinical states of water diuresis and deprivation, salt ingestion and deprivation, *diabetes insipidus* and the exhibition of antidiuretic hormone, Addison's disease and the exhibition of adrenal cortical hormone, cardiac and nephritic œdema and the action of mercurial diuretics. Very close qualitative agreement between the response of the model and the clinical findings is reported.

The implications of the hypothesis and the gaps in our knowledge of the physiological processes involved are discussed.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the assistance given to this investigation by many colleagues and by the staff of the Baker Medical Research Institute and Alfred Hospital Clinical Research Unit. In particular I thank Mr. J. J. Robinson for his help in constructing the model.

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ISONIAZID TREATMENT OF PULMONARY TUBERCULOSIS IN OUT-PATIENTS: A CLINICAL TRIAL[1]

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Soon after the first reports appeared, early in 1952, of the use of isonicotinic acid hydrazide (isoniazid) in pulmonary tuberculosis (Robitzek, Selikoff and Ornstein, 1952), a supply of the drug was made available to us for therapeutic trials. It was decided to conduct a strictly controlled clinical trial in the class of patients which forms an important part of the work of the pulmonary clinic of this hospital—out-patients with chronic pulmonary tuberculosis. It was with ambulatory patients with chronic disease that we were concerned—patients who are not sufficiently ill to be admitted to hospital as suffering from acute disease, and who act as mobile sources of infection in the community. Many of the patients had been in sanatoria and had been treated with streptomycin and PAS, and still presented a therapeutic and social problem.

The main question was whether isoniazid was of any value in the treatment of this type of patient; and since there was initially very little information about the best way to use isoniazid and the effects of usage, and since the number of patients available for the trial was limited, it was decided to test the effect of isoniazid given alone. When it became known in the following year that it was preferable to give isoniazid in combination with other chemotherapeutic agents (Medical Research Council, 1953, *b*, *d*) the trial was closed. Thus the trial was so designed that some patients were given isoniazid while others were given inert placebo tablets, and neither patient nor physician knew which tablets were being dispensed. In this way the patient's symptoms and the physician's impressions as well as objective measurements could be used for assessing the effectiveness of treatment. Treatment was given for two or four months. An account of the conduct and result of this trial follows.

[1] Received September 10, 1954.

Plan and Conduct of the Trial

Selection of Patients

All patients who conformed to certain criteria and attended the pulmonary clinic of this hospital between June, 1952, and October, 1953, were entered in the trial. The criteria were as follows: the patients, male or female, had to be aged forty years or more, suffering from chronic pulmonary tuberculosis, with positive findings in the sputum or on gastric lavage, X-ray evidence of unilateral or bilateral fibrocaseous lesions with multiple cavitation, no evidence of pneumonoconiosis, and having received no drug therapy within the previous three months.

Treatment

The allocation of patients to treatment or control groups was determined by reference to prearranged lists based upon random sampling numbers. The lists were divided according to the sex and age (under fifty years, or fifty years and over) of the patient and whether or not the patient had ever been treated with streptomycin or PAS. Neither patient nor physician knew to which group any patient was allotted. Patients in the treatment group were given isoniazid tablets for a period of four months in a dosage determined by body weight—four tablets (200 milligrammes) for a weight of 9 to 10 stone (56 to 62 kilograms), five tablets (250 milligrammes) for 10 to 11 stone (62 to 68 kilograms), and six tablets (300 milligrammes) for heavier patients—divided into three or four doses per day. Patients in the control group were given inert tablets indistinguishable in appearance from the isoniazid tablets in the same sliding scale of dosage. After two months in the trial some of the patients in the control group, selected again by reference to prearranged lists, were changed over to isoniazid tablets for the second two-month period of the trial.

At the conclusion of the four months' trial the patient's progress was discussed by the physicians of the clinic. The treatment the patient had received was then revealed and future treatment was decided upon. Most of the patients who had initially entered the control group were given isoniazid in this extra-trial period.

Observations

The patients attended weekly and then fortnightly. Records were made fortnightly of the patient's own assessment of changes in his general condition—cough, dyspnœa, hæmoptysis, pain in the chest, night sweats—of the physician's impression of change in the condition, and of any possibly toxic effects. The urine was tested at each visit, and measurements were made of body weight, hæmoglobin value of the blood, the white cell count and the sedimentation rate (Westergren). A twenty-four hour collection of sputum was measured, smears were examined for the presence of acid-fast organisms, cultures were made and sensitivity of the organisms to isoniazid was measured. Patients who were admitted to the trial with negative sputum findings but positive findings on gastric lavage had the lavage repeated at the end of the two or four months' treatment. Full-plate chest X-ray films were taken monthly.

Bacteriological tests were carried out as follows:

Microscopic Examination. — Films of untreated sputum were stained by Ziehl-Nielsen's method and examined by one observer with a one-seventh inch oil immersion objective lens. The bacterial density was assessed according to the Gaffky scale (Meakins, 1950), which has ten categories based on the average number of bacilli seen in each microscopic field.

Culture and Sensitivity in Vitro.—Löwenstein-Jensen medium was prepared according to the standard method. A stock solution of isoniazid in distilled water was sterilized by boiling for ten minutes, and a constant volume of appropriate dilutions of this stock solution was added to the medium before inspissation to give the required concentrations. Early in the trial the concentrations used were 0·0, 0·01, 0·1 and 1·0 microgramme per millilitre. Later, concentrations of 5, 10, 20 and 50 microgrammes per millilitre were added and the 0·01 microgramme sample was omitted. The media were sloped in Macartney's screw-capped bottles and stored in the refrigerator. Fresh batches of media were prepared at two-monthly intervals and checked against strains of bacilli whose sensitivity had been determined previously. The total measured volume of sputum was digested with 3·8% sulphuric acid and centrifuged. A constant amount of the deposit was inoculated directly onto two bottles of the drug-free medium and onto one set of bottles containing the range of concentrations of isoniazid. These were incubated and examined weekly for eight weeks. If macroscopic growth appeared in four weeks or less, the cultural finding was regarded as positive; if this growth occurred on media containing one microgramme or more of isoniazid per millilitre, the organism was classified as resistant.

X-Ray Examinations.—X-ray films of each patient taken before and after the various periods of treatment for two or four months were placed before each of six observers, three radiologists and three physicians. Without having any knowledge of the patient's name or treatment, they recorded their opinions as to whether there was improvement, no change, or radiological deterioration. The average of the opinions expressed at their first viewing of the films has been taken as the true assessment for the purpose of this study.

PATIENTS IN THE TRIAL

Thirty-one patients who satisfied the criteria for selection attended the clinic and were enrolled in the trial. Five of these patients were subsequently excluded—one died suddenly soon after enrolment, one was admitted to a sanatorium, one defaulted, and two were inadvertently given ordinary isoniazid from the dispensary (one of these provided data incorporated in the extra-trial results; the other attended irregularly).

Twenty-six patients completed two months or more of the trial. All but two were males, with an average age of fifty-four years. Thirteen received isoniazid for the first two months, and 11 of these continued for four months (one moved residence, the other defaulted) and 13 had placebo tablets for two months. Six of the latter continued with the placebo for the full four months, five were changed to isoniazid, and two were excluded from the trial (one was given streptomycin and PAS because of deterioration, and the other changed to isoniazid, but treatment was stopped because of severe diarrhœa thought to be a toxic effect of the drug). At the conclusion of the trial four of the six patients who had formed the control group took isoniazid for the next four months and four of the five who had changed to isoniazid for the

second half of the trial continued with the drug to complete four months of treatment.

For the purpose of assessing the results of treatment we are able to group the patients in different ways. First, taking only the data collected during the trial proper, we can compare the results in the following groups: (*a*) 13 patients who had treatment during the first period of two months of the trial, and 13 patients who were controls during that period; (*b*) five patients who had treatment in the second period after being controls in the first, and four patients who were controls in the second period after a similar experience in the first (the other two controls had become bacteriologically "negative"); (*c*) a combination of (*a*) and (*b*)—that is, 18 treated patients and 17 controls for a period of two months; although some patients appear twice in these groups, different

TABLE I

The Number of Patients in the Various Groups Mentioned in the Text

Group	Period of Observation (Months)	Treatment Series	Control Series
In trial only:			
(*a*)	2 (first)	13	13
(*b*)	2 (second)	5	4
(*c*)	2	18	17
(*d*)	4	11	6
In and out of trial:			
(*e*)	2	23	17
(*f*)	4	20	6

sets of data are involved and the comparisons are legitimate; (*d*) for a comparison on the basis of four months' therapy there are 11 treated and six control patients. Secondly, data collected outside the trial proper—that is, while ordinary isoniazid was being prescribed and taken knowingly—can be used to supplement the data from the trial in so far as the assessment of objective data is concerned. The addition of these patients to those of groups (*c*) and (*d*) above gives (*e*), a group of 23 patients treated for two months, and (*f*) 20 patients treated for four months. These groups are shown in Table I. Groups (*c*) and (*d*) have been used for assessing the effect of treatment on the patients' symptoms and the clinical impressions of the physicians, and groups (*e*) and (*f*) have been used for the assessment of objective results. Results have been regarded as significant when the probability of their occurring by chance is less than 5%. The ages and initial state of the patients in the various treated and control groups were comparable; the main features are listed with the results of the trial.

RESULTS

Patients who were treated with isoniazid for a period of two months showed definite improvement in blood sedimentation rate, radiological appearances and bacterial content of sputum; but these benefits were reduced or lost when treatment was continued for four months and bacterial resistance had

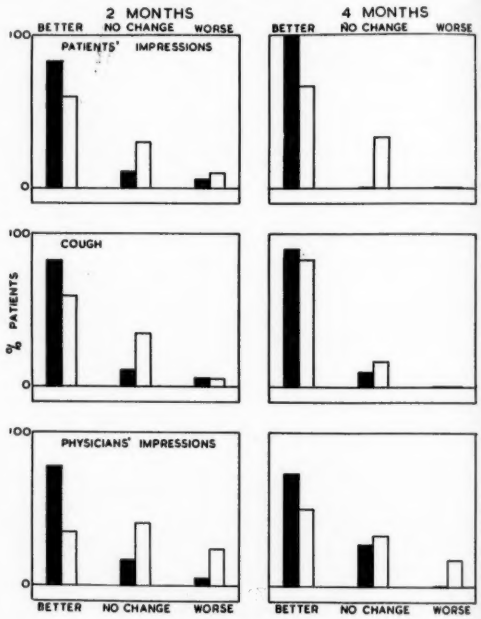

FIGURE I

Subjective changes in the treated (black columns) and control (open columns) groups

increased. The physicians' opinions of the progress made by these patients were in accord with these findings. In general there was no improvement attributable to treatment in relation to the patients' symptoms, body weight or volume of sputum.

Symptoms and Impressions

A majority of the patients in both groups expressed the opinion that their general condition had improved and their cough had diminished during the trial. Figure I shows the percentages of patients in each group who believed they had improved, remained unchanged or deteriorated during the short

TABLE II

Clinical Impressions of the Physicians Related to Other Observations Recorded During Two Months of Trial. Figures Refer to Number of Patients, Treated and Controls Combined

TABLE IIA

Physician's Opinion	Blood Sedimentation Rate[1]			Body Weight[2]		
	Improved	No Change	Worse	Improved	No Change	Worse
Improved	8	12	0	6	14	0
No change	1	7	2	1	8	1
Worse	0	3	2	2	1	2

[1] Changes of 10 millimetres or more in one hour; $P=0{\cdot}05$ to $0{\cdot}02$.
[2] Changes of 1·5 kilograms or more; $P=0{\cdot}05$ to $0{\cdot}02$.

TABLE IIB

Physician's Opinion	Patient's Opinion[1]			Organisms in Smear of Sputum[2]	
	Improved	No Change	Worse	Present	Absent
Improved	20	0	0	2	18
No change	4	5	1	5	5
Worse	1	3	1	4	1

[1] $P<0{\cdot}001$.
[2] $P=0{\cdot}01$ to $0{\cdot}001$.

(two-months) and long (four-months) periods of the trial. More of the treated than of the control group recorded improvement, but the difference is not significant. In spite of this apparent lack of effect of isoniazid on the symptoms of patients considered as a group, the response of two individuals was dramatic. Both had been observed as controls for a period, and both lost their cough and were unable to produce any sputum within a week or two of changing to isoniazid. Neither group recorded any changes in the incidence of hæmoptysis, dyspnœa, pain in the chest or sweating at night. Five patients in the treated group had minor complaints which were thought to be due to toxic effects of the drug—muscular twitching and depression, drowsiness, headache and a sense of fullness of the eyes, diarrhœa and sore tongue.

The physicians who were observing the patients were impressed by improvement in the clinical condition of a greater number of patients in the treated group than among the controls. The difference between the groups, illustrated in Figure I, is significant. These opinions were expressed without the knowledge of whether the patient was having treatment or not; but the results of blood tests and examinations of smears of sputum were available. It is interesting therefore, though not surprising, to find a very definite agreement between the opinions given by the physicians and the changes observed in the blood sedimentation rate, the presence or absence of organisms in the sputum, the changes in body weight and the opinions expressed by the patients themselves. These relationships for the two-months period of the trial are shown in Table II, and serve to illustrate some of the factors which influence the formation of "clinical impressions".

Body Weight

Treatment with isoniazid did not influence body weight. The average increases in weight of the patients treated for two and four months were 0·5 kilogram and 0·7 kilogram respectively. The corresponding figures for the control patients were 0·6 kilogram and 1·1 kilograms. The proportion of patients in the treated group who gained weight and the extent of their gains were the same as in the control group.

Sedimentation Rate

Table III and Figure II show the changes in blood sedimentation rates. The control group of patients showed no significant alterations during the trial. The treated group, however, showed a definite improvement in the first two months of treatment, but this was lost when treatment was continued for four months. Thus the number of treated patients with a blood sedimentation rate of more than 20 millimetres in one hour was nine before treatment was begun, two after two months, and five after four months. Looking at the results

from the viewpoint of individuals instead of groups, the blood sedimentation rate improved by 10 millimetres or more in nine (39%) of the patients treated for two months and became

TABLE III

Changes in Sedimentation Rates in the Control and Treated Groups

Group	Blood Sedimentation Rate (Millimetres in One Hour)		
	0 to 20	21 to 50	51 and Over
Control :			
Initially	11	5	1
After two months ..	12	3	2
Treated :			
Initially	14	8	1
After two months ..	21	1	1
Control :			
Initially	3	3	0
After four months ..	4	2	0
Treated :			
Initially	11	9	0
After four months ..	15	5	0

worse in none, while it improved in only 25% of patients treated for four months and became worse in 20% (Figure II).

This initial improvement and later deterioration in blood sedimentation rate is further

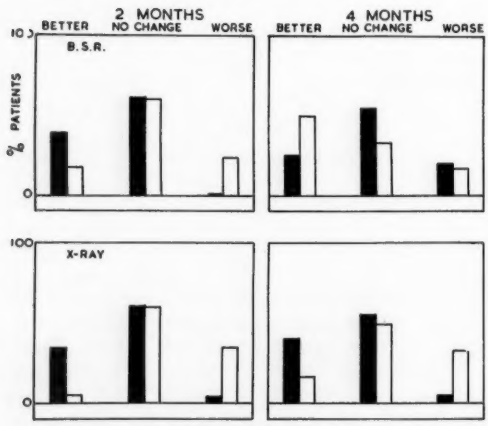

FIGURE II

Changes (of 10 millimetres or more in one hour) in the blood sedimentation rates and radiological appearances in the treated (solid columns) and control (open columns) groups

illustrated by the experiences of the 20 patients who received isoniazid for four months. During the first half of treatment nine showed improvement and none became worse, but during the second half one improved while five became worse.

Our results showed a significant correlation between change in blood sedimentation rate and the physician's impression of change in clinical condition for both the short and long periods of the trial (Table II). There was no significant agreement between change in blood sedimentation rate and changes in weight, in X-ray appearances, in bacterial content of the sputum or in sensitivity of the organisms to isoniazid.

Radiological Appearances

There was a significant radiological improvement in the treated patients which was not observed in the controls. At the end of two months there was radiological improvement in

TABLE IV

Radiological Appearances in 20 Cases After Four Months' Treatment

Period	Improved	No Change	Deteriorated
In two months.. ..	7	12	1
In four months ..	8	11	1

eight of 23 treated patients and deterioration in one, while only one of the 17 controls showed improvement and six had deteriorated. After four months there was improvement in eight of 20 treated patients and deterioration in one, and improvement in one of the six controls, with deterioration in two. These results are illustrated in Figure II.

TABLE V

Changes in Body Weight Related to Changes in Radiological Appearances

X-ray Appearances	Body Weight[1]		
	Improved	No Change	Worse
Improved	3	5	1
No change	7	17	0
Worse	0	4	3

[1] Changes of 1·5 kilograms or more; P=0·02 to 0·01.

A comparison of the results at two months with those at four months in the 20 patients observed over the full course of treatment suggests that most of the radiological improvement occurred in the first two months (Table IV).

Six of the patients showing improvement at the end of four months had already shown improvement in two months.

There was no significant correlation between radiological change and blood sedimentation

rate, bacteriological findings or the clinical impression of the physician. There was, however, a definite relation between change in radiological appearance and change in weight (by 1·5 kilograms or more), an example of which appears in Table V.

Sputum

Volume.—Little reliability can be placed on the collection by outpatients of twenty-four-hour accumulations of sputum, and the amounts returned by our patients were very variable. The amount of sputum did not change significantly throughout the trial (Table VI).

TABLE VI

The Numbers of Patients Producing Different Quantities of Sputum Before and After Two Months in the Trial

Group	Volume of Sputum (Millilitres in Twenty-four Hours)			
	Nil	1 to 20	21 to 40	>40
Control :				
Initially.. ..	0	11	4	2
After two months	1	11	2	3
Treated :				
Initially.. ..	0	13	4	6
After two months	1	12	3	7

Bacterial Content.—Treatment with isoniazid caused a marked reduction in the bacterial content of the sputum. Table VII shows that the results of cultural examination of the sputum became negative in 13 of 23 patients treated for two months and in 12 of the 20 patients treated for four months—that is, in 50% to 60% of cases. In addition, treatment caused a significant reduction in the numbers of bacilli seen in samples of smear-positive sputum, as shown in Table VIII and Figure III. Changes in the control group were not significant, but it is interesting to notice that improvement occurred in some of the patients taking inert tablets.

TABLE VII

The Results of Attempted Culture of Sputum

Group	Culture		Organism	
	Growth	No Growth	Sensitive	Resistant
Control :				
Initially.. ..	15	2	14	1
After two months	13	4	9	4
Treated :				
Initially.. ..	23	0	19	4
After two months	13	10	8	5
Control :				
Initially.. ..	6	0	6	0
After four months	4	2	2	2
Treated :				
Initially.. ..	20	0	17	3
After four months	12	8	4	8

The effects of treatment for four months were not so favourable as for two months. This is seen most clearly by examining the results at

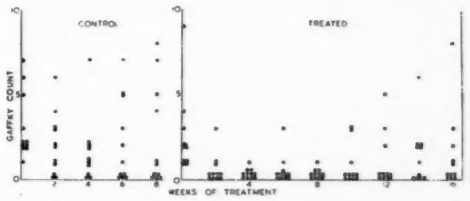

FIGURE III

Fortnightly estimates of bacterial density in the sputum of 11 control and 11 treated patients

different stages of treatment of the 20 patients who took isoniazid for four months. Figure IV and Table IX show these results. The number of patients with positive findings on sputum

TABLE VIII

The Effect of Treatment on the Bacterial Content of Sputum

Group	Culture		Growth or No Growth on Culture : Gaffky Scale			
	No Growth : Gaffky Scale 0	Growth : Gaffky Scale 0	1 to 2	3 to 5	6 to 7	8 to 10
Control :						
Initially	0	5	7	3	2	0
After two months ..	3	7	3	2	1	1
Treated :						
Initially	0	4	10	4	2	3
After two months ..	9	12	2	0	0	0
Control :						
Initially	0	1	4	1	0	0
After four months ..	2	3	1	0	0	0
Treated :						
Initially	0	3	10	4	1	2
After four months ..	8	5	4	2	0	1

culture was 20 initially, 12 at two months and 11 at four months. Organisms were seen in a smear of sputum from 17 patients before treatment, from only one at the end of two months and from eight after four months.

Isoniazid Sensitivity

Accepting the criteria mentioned earlier and paying attention only to those tests of sensitivity made before the patient entered the trial and at the ends of the specified periods (disregarding the results of tests made at other visits), we have the results shown in Table VII. The most prominent feature is the increase in the number of patients with resistant organisms after treatment for four months. The true significance of this is obscured, however, by the fact that one patient in the control group, who had never taken isoniazid, had a resistant organism before entering the trial, and three other patients showed resistant organisms after taking the inert tablets for two or four months.

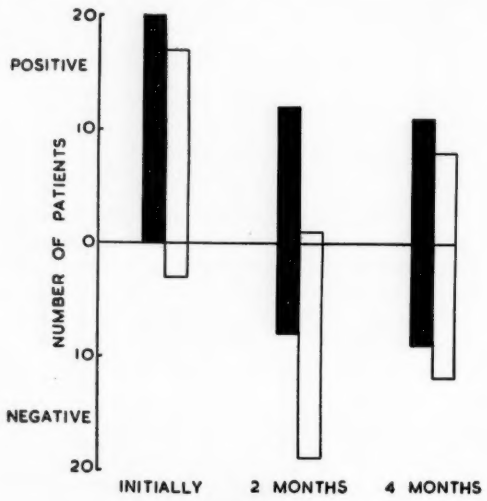

FIGURE IV

The results of cultures (solid columns) and of examinations of smears of sputum (open columns) at different stages during treatment

The development of resistance during treatment with isoniazid is supported by the observations given in Table IX, which shows the increasing incidence of resistant organisms among the 20 patients who were treated for four months. Becoming resistant to isoniazid, at least according to the criteria adopted here, does not prevent the organism disappearing from the sputum when treatment is continued. The sputum of all three patients who had resistant organisms before starting treatment gave negative cultural results within two months, and one of the four who developed resistant organisms during the first period of treatment gave negative cultural results in the second period. Equally interesting is the observation that organisms reappeared in the sputum of two of the eight patients who had given negative cultural results at two months, but in both instances the organisms were sensitive, not resistant.

DISCUSSION

The type of patient with whom this trial has been concerned, the middle-aged patient with chronic pulmonary tuberculosis, shows definite evidence of improvement when treated with isoniazid; but this improvement tends to be lost as treatment is continued. These results are substantially the same as those observed in a therapeutic trial among outpatients by Joiner *et alii* (1952, 1953) and in the patients of Group 3 with chronic disease studied by the Medical Research Council of Great Britain (Medical Research Council, 1952, 1953*a*). Treatment for two months produced in a significant number of cases an improvement in the blood sedimentation rate and in radiological appearances, and a reduction in the bacterial content of sputum, but there was no worthwhile change in symptoms, volume of sputum or body weight. The results after treatment for four months still showed an

TABLE IX

Changes in the Bacterial Content of Sputum and in Isoniazid Sensitivity During Treatment with Isoniazid for Four Months

Time of Test	Culture		Growth or No Growth on Culture: Gaffky Scale				Organisms Sensitive	Organisms Resistant
	No Growth: Gaffky Scale 0	Growth: Gaffky Scale 0	1 to 2	3 to 5	6 to 7	8 to 10		
Initially	0	3	10	4	1	2	17	3
After two months ..	8	11	1	0	0	0	8	4
After four months ..	7	5	5	2	0	1	4	7

improvement, radiological and bacteriological, on the pre-treatment findings; but there was evidence of regression in the sedimentation rate and in the bacterial content of the sputum in the latter part of the trial. This deterioration in clinical condition which occurs as treatment is prolonged is associated with an increasing development of organisms resistant to isoniazid.

The greatest handicap to the successful treatment of tuberculosis with drugs is the development of bacillary resistance. In our small series, 20% of patients treated with isoniazid were excreting resistant organisms after two months and 40% after four months. However, not all the effects of isoniazid are to its discredit. Another 40% of patients had become "bacteriologically negative" with both the short and longer periods of treatment. Moreover, the assessment of bacterial resistance is by no means straightforward (as the comments made later in this report illustrate). Some patients harboured resistant organisms at the commencement of treatment, but the presence or development of resistance did not preclude the possibility that the sputum might be sterilized by further treatment. Furthermore, there is good evidence that tubercle bacilli which are resistant to isoniazid are less virulent than normal (Mitchison, 1954). The fact remains, however, that the development of bacillary resistance is associated with deterioration in clinical condition (Medical Research Council, 1952) and should be avoided if possible. Of the greatest importance, therefore, was the demonstration that the emergence of bacterial resistance is greatly reduced when isoniazid is given with streptomycin (Medical Research Council, 1953*d*) or with PAS (Medical Research Council, 1953*b*), making it clear that isoniazid should not be given alone. It was for this reason that our trial was brought to a close.

Perhaps the most interesting result of our trial lies in the significant differences observed between the treated and the control subjects, in spite of the small number of patients involved. This is a tribute to the potency of isoniazid as much as to the usefulness of the controlled clinical trial as a technique for measuring the effectiveness of treatment. Trials which include small numbers of patients cannot be expected to produce such definite conclusions or answers to such a variety of questions as might be given by large-scale trials; but, as has been emphasized elsewhere (Leading Article, 1952), they may serve a useful purpose in providing pointers and in making a more detailed study of some aspects of treatment. We are particularly fortunate with regard to tuberculosis in having a highly efficient service of investigation provided by the Medical Research Council of Great Britain. Almost any other field offers even greater opportunities for the small clinical trial which, if it provides nothing more, will give factual substance to what might otherwise have been but vague impressions.

Comments on Bacteriological Technique

The sputum of tuberculous patients probably contains a very mixed assortment of organisms, which vary widely in their viability, growth requirements and sensitivity to isoniazid. This variation is increased by treatment, and the number of organisms present in sputum with any particular characteristic is likely to vary from time to time. Variations such as these help to explain, at least in part, some of the bacteriological anomalies encountered in this study. For example, of 132 tests of sputum from patients having treatment, in smears from which organisms were seen, 27 produced no growth in attempted culture on drug-free media. Similar findings have been reported by others (Medlar, Bernstein and Steward, 1953; Collard *et alii*, 1953; Ogilvie, 1953). Eight of these 27 specimens showed growth on media containing isoniazid, even in high concentration, which is suggestive of the development of isoniazid dependence (Bryson *et alii*, 1953). In some other tests in which growth occurred on drug-free media, growth also occurred on media containing as much as 50 microgrammes of isoniazid per millilitre, but not on media with lower concentrations.

Because the organisms in sputum are not uniform in their susceptibility to isoniazid, the Medical Research Council (1952, 1953*c*) required 20 or more colonies to be present before accepting a culture as positive. Our criterion was any extent of growth which was macroscopically visible, and therefore our results include more positive cultures than the Medical Research Council standards would allow. In other respects—length of incubation and the lowest concentration of drug which allows growth of a sensitive organism—our criteria conform with the Medical Research Council recommendations. How the results would be affected by accepting different periods of incubation is shown in Table X. Here the figures include the results of the last sputum test in each month of the trial from patients who had regular tests, from control patients, and from those treated patients who showed resistant organisms at some time during treatment. Obviously there is little advantage

in prolonging incubation from one to two months; but less than half the colonies would be reported as "positive" were the cultures to be read after incubation for only two weeks. There was no apparent retardation of growth among the resistant organisms, as they responded to an increased incubation period to the same extent as the bacilli from control patients.

The effect of length of incubation on the estimation of resistance is more important. There appears to be a direct relation between the duration of incubation and the number of tests which show growth on media containing 1·0 microgramme or more of isoniazid per millilitre. There were 10 "resistant" growths after incubation for two weeks, 27 after four weeks and 55 after two months. The overall incidence of resistance was greater among

TABLE X

The Numbers of Tests Showing Growth and Resistance after Different Periods of Incubation in Selected Cases in the Control and Treated Groups

Length of Incubation (Weeks)	Culture Positive		Resistance	
	Control Group	Treated Group	Control Group	Treated Group
2	16	18	1	9
4	25	48	6	21
8	27	54	11	34

treated than among control patients, but the influence of length of incubation was the same in both groups. A likely explanation is that the potency of isoniazid in Löwenstein-Jensen medium deteriorates during incubation in the same way as it has been found to do in Dubos' liquid medium (Knox *et alii*, 1952; Mitchison, 1952). Some observations made by transferring sensitive organisms to isoniazid-containing media which had previously been incubated for varying lengths of time support this hypothesis, but further experiments are required. If this is true, then additional importance attaches to any variation which exists among the organisms in the sputum. Sensitive organisms will be killed by isoniazid in the culture media. The most resistant ones will grow in spite of the drug. Some of the less resistant ones, however, may manage to survive until the drug deteriorates sufficiently to allow them to grow, and these will produce their colonies later. Increasing resistance of organisms will result in growth occurring progressively earlier. Examination of the monthly data included in Table X showed that this did apply to the treated series (P=0·01 to 0·001), but not to the control series, in which bacterial resistance frequently was feeble and inconstant.

ACKNOWLEDGEMENTS

We are grateful to other members of the pulmonary clinic for clinical assistance, to Dr. J. N. Sevier, Dr. A. Hogan, Dr. M. F. Deck, Dr. J. Raftos, Dr. K. W. Bretherton and Dr. L. C. L. Lawrence, of the Tuberculosis Division of the Department of Health, and to Sister I. Joyce and Sister J. Sinclair, without whose help the trial could not have been made. Radiologists who assisted in assessing the radiological changes were Dr. H. M. Cutler, Dr. D. G. Maitland and Dr. H. G. Marsh. Frederick Stearns and Company specially prepared and kindly donated all the isoniazid and placebo tablets used in the trial.

SUMMARY

The conduct and results of a clinical trial are described, in which some patients were treated with isoniazid for two or four months and others were given inert placebo tablets. All patients were suffering from chronic pulmonary tuberculosis and attended the out-patient department.

Treatment with isoniazid caused a substantial improvement in the clinical condition of patients, as judged by the impressions of the physicians, blood sedimentation rate, radiological appearances and bacterial content of the sputum. There was some regression when treatment was continued for four months, and bacterial resistance increased.

Some of the difficulties encountered in the bacteriological studies are discussed.

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ASPECTS OF COPPER METABOLISM IN WILSON'S DISEASE[1]

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HEPATOLENTICULAR DEGENERATION, first described by Kinnier Wilson in 1912, is an uncommon disease (only one other case has hitherto been diagnosed in this State, with a population of 600,000), characterized by the clinical association of cirrhosis of the liver, neurological symptoms and signs attributable to degenerative changes in the lenticular nuclei (muscle rigidity, dysarthria, athetoid movements and emotional lability), and the appearance of a peripheral rim of greenish-brown corneal pigmentation (Kayser-Fleischer rings). A comprehensive study of the pedigrees of many patients has produced evidence that the disease is inherited as an autosomal recessive (Bearn, 1953). That a disorder of copper metabolism plays an important role in the pathogenesis of Wilson's disease was evident from the finding by Glazebrook (1945) of a high copper content of both brain and liver, and by Mandelbrote and his colleagues (1948) of a high level of excretion of copper in the urine. Amino-aciduria was discovered by Uzman and Denny Brown (1948) to be a further detectable biochemical anomaly. Since an increase in the level of serum amino acids has not been found in cases of Wilson's disease (Cooper *et alii*, 1950), defective tubular reabsorption of amino acids must be presumed, and in this respect Wilson's disease falls into line with other recently identified familial aminoacidurias (see review of Brick, 1952).

It is a progressive fatal disorder, death occurring in the second or third decade usually from the complications of cirrhosis. Mobilization of copper from the brain and the liver has been a major object of treatment. BAL (2 : 3 dimercaptopropanol) was used as the copper-mobilizing agent by Denny Brown and Porter (1951), who noted a striking improvement in the neurological signs in many of their patients treated with short courses of the drug. Calcium ethylenediamine tetra-acetate (" Versene ") is a recently discovered powerful chelating agent (Sidbury *et alii*, 1953); its use in the treatment of Wilson's disease has not been previously reported.

In this communication the clinical, pathological and biochemical features of a typical case of hepatolenticular degeneration are described. The mobilization of copper following the administration of BAL and " Versene " has been studied quantitatively, and a full copper balance study has been carried out over a thirteen day period. An attempt has been made to ascertain whether chelating substances are capable of removing more copper than gains entrance to the body under ordinary physiological circumstances.

METHODS OF STUDY

Tests of liver function and of serum electrolytes were performed by standard laboratory techniques.

Urinary amino acid nitrogen was estimated by the method of Peters and Van Slyke (1932), modified by Frame *et alii* (1943). Individual amino acids were identified by filter paper chromatography.

The copper content of urine, blood, tissues and food was determined by the diethyl-dithiocarbamate method after wet digestion of the material with nitric, sulphuric and perchloric acids (Eden and Green, 1940). All specimens were collected in stainless steel metal containers, or in glass washed in acid and glass-distilled water.

During the thirteen days of the copper balance study the patient received a diet to which he was accustomed, containing approximately 150 grammes of protein and 500 milligrammes of sodium per day. Much of this protein was provided in the form of milk foods reinforced with proprietary protein hydrolysates. All food was weighed and aliquots were taken for copper analysis. Returned food was weighed and analysed.

CASE REPORT

A young man, aged twenty-two years, was first admitted to the Royal Perth Hospital for investigation in March, 1953. No family history of Wilson's disease was elicited. As a child the patient had been regarded as delicate, being subject to frequent bilious attacks.

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His scholastic achievement was slightly above average. At the age of sixteen years he was noted to be jaundiced and to have moderate œdema of the ankles. The jaundice persisted for many months and was attributed to chronic infectious hepatitis. A year later he began to feel tired and became conscious of slight clumsiness of his fingers, finding difficulty in doing up his buttons. Slight indistinctness and monotony of speech were also commented on at this time by close relatives. He was of a cheerful disposition, and was able, despite these minor disabilities, to do part-time work as a reporter.

In May, 1952, he had an attack of pneumonia. It was noticed then that his dysarthria was considerably worse. He had become aware of increasing stiffness of gait and of a tendency for saliva to trickle from the angles of his mouth.

On admission to hospital the patient was noted to be a youthful looking man, with a smooth chin and pronounced facial acne. He had a fixed smile exposing the upper teeth. Axillary and suprapubic hair was scant. Bilateral gynæcomastia was present; numerous spider nævi were present over the arms and chest. The scleræ were slightly icteric. A rim of greenish-brown pigmentation (Kayser-Fleischer rings) was present in the limbus of each cornea. On the skin of the legs and other exposed areas pronounced melanotic pigmentation was seen. Pitting œdema was present below both knees. The heart and lungs were normal. The spleen was palpated five centimetres below the left costal margin, but the liver could not be felt and the area of hepatic dullness was diminished. Generalized plastic hypertonia was present. Pronounced tremor of pill-rolling type was present, made worse by voluntary movement and emotional excitement. His speech was monotonous and indistinct, sentences tending to tail off into inaudibility. There was a notable poverty of facial expression, the fixed smile being unwavering. Choreiform or athetoid movements were not a feature of his illness. His gait was of shuffling character. The deep reflexes were all brisk and the plantar responses were flexor. There were no signs of sensory loss.

Investigations

The results of investigations were as follows. The hæmoglobin value was 15·1 grammes *per centum*; the leucocytes numbered 5100 per cubic millimetre. The fasting blood urea content was 38 milligrammes per 100 millilitres; the result of the urea clearance test was 110% of normal. The urinary excretion of 17-ketosteroids was five milligrammes per twenty-four hours; the total urinary uroporphyrin excretion (method of Sveinsson *et alii*, 1949) was 6·0 micrograms per twenty-four hours (normal, nil to a trace); the total coproporphyrin excretion (method of Schwartz *et alii*, 1951) was 32·9 micrograms per twenty-four hours (normal, 52 to 250 micrograms). The total amino acid nitrogen excretion was 0·615 gramme per twenty-four hours.

In six controls the range of total amino acid nitrogen was from 0·282 to 0·504 gramme per twenty-four hours, with a mean of 0·352 gramme. The following amino acids were identified by filter paper chromatography: ? aspartic acid " + ", ? glutamic acid " + ", cystine " +++ ", glycins " +++ ", lysine " +++ ", alanine " ++++ ", tyrosine " + ", valine " ++ ", methionine " ++ ", leucine " trace ".

Serial estimations of tests of liver function and serum electrolyte contents were performed (see Table I). The most notable abnormalities seen were the reversal of the albumin-globulin ratio, a rising and falling positive thymol flocculation, and slight hyperchloræmic acidosis with hypokalæmia. A bromsulphthalein excretion test on one occasion showed 21% retention of dye at forty-five minutes.

A barium bolus X-ray examination of the œsophagus failed to reveal the presence of œsophageal varices.

Treatment and Progress

The downhill progress of the patient was observed in six prolonged periods in hospital.

March-April, 1953.—During the period March and April, 1953, preliminary observations were carried out on the patient, and a diet of high protein and low sodium content was given to control the œdema.

June-July, 1953.—By June, 1953, the œdema had almost completely subsided. His speech was noticeably less distinct and his gait more strikingly shuffling in character. On this occasion a ten-day copper balance study was performed prior to a first course of treatment with BAL. The regime recommended by Denny Brown and Porter (1951) was carried out as follows: two millilitres of a 10% solution of BAL in peanut oil were given intramuscularly twice daily for ten days. There was no noticeable improvement in his neurological signs during or after this course of treatment.

TABLE I

Results of Serial Tests of Liver Function in a Case of Wilson's Disease. Courses of BAL were Given in June and August, 1953. In October, 1953, a Course of " Versene " was Given

Date	Serum Bilirubin Content (Normal, <0·5 Milligrammes per 100 Cubic Millimetres)	Serum Alkaline Phosphatase Content (Normal, 3 to 13 King-Armstrong Units)	Thymol Turbidity (Normal, 0 to 3 Units)	Thymol Flocculation (Normal, Nil)	Zinc Sulphate Turbidity (Normal, 0 to 6 Units)	Serum Albumin Content (Normal, 3·5 to 5·0 Grammes per 100 Millilitres)	Serum Globulin Content (Normal, 2·0 to 3·5 Grammes per 100 Millilitres)	Serum Sodium Content (Normal, 140 to 152 Milliequivalents per Litre)	Serum Potassium Content (Normal, 4·0 to 5·2 Milliequivalents per Litre)	Serum Chlorine Content (Normal, 98 to 106 Milliequivalents per Litre)	Serum Carbon Dioxide Content (Normal, 23 to 34 Milliequivalents per Litre)
10. 3.53	1·6	15·7	2	0	7	2·7	4·0			106	20·0
9. 4.53	1·0	15·2	2	++	5	3·1	3·1	148	3·1	111	21·0
5. 6.53	1·0	21·6	2	+	7	2·8	3·2				
3. 7.53	1·1	16·9	2	+	6	3·3	2·9				
15. 7.53	0·4	18·1	3	+++	8	4·1	3·3				
31. 7.53	1·3	23·2	2	+++	9	4·4	3·1				
7. 8.53	1·4	17·1	2	++	9	4·2	2·8				
23.10.53	0·2	9·2	2	0	6	2·1	3·1	146	2·9	107	17·0
22. 4.54	4·0	15·7	2	0	11	1·4	3·9	139	3·0	109	21·5
27. 4.54	—	—	—	—	—	1·2	3·7				

August, 1953.—In August, 1953, he was given a second course of treatment with BAL. All observers agreed that far from improving, he began to deteriorate at this time. His speech became less distinct, his tremor more agitated and his gait more shuffling, and in addition he became emotionally excited. He roamed the wards all night and slept little during the day. It became necessary to transfer him to a mental institution, where unfortunately facilities for giving him a special diet were not available.

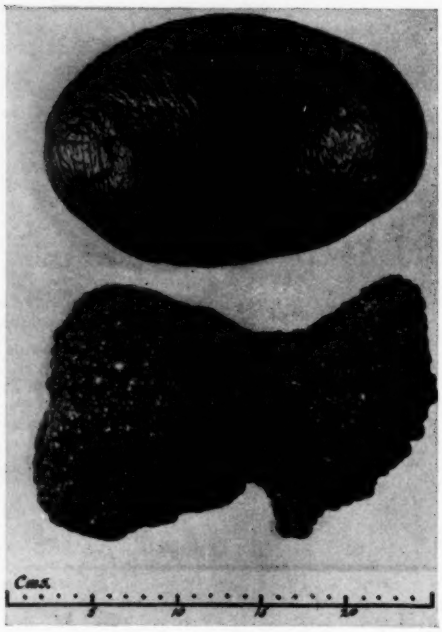

FIGURE I

Atrophic cirrhosis and congestive splenomegaly in Wilson's disease

October, 1953. In October, 1953, his equanimity had returned. A staphylococcal infection of the leg was associated with high fever. He became drowsy and was readmitted to the Royal Perth Hospital in a semi-comatose state. Œdema had reaccumulated. With penicillin treatment he improved, and after a period of normal dietary intake a six-day course of "Versene" (calcium ethylenediamine tetraacetate) was given. One gramme was given intravenously over a period of two hours each day. On the fourth day he developed a purpuric rash on the right forearm, and had three small epistaxes. No alteration was noted in the bleeding or clotting times or in the level of circulating platelets, but the serum calcium content was found to have fallen from 9·4 to 8·4 milligrammes per 100 millilitres; calcium gluconate was given intramuscularly. The two remaining daily doses of "Versene" were given without further complications. No objective signs of improvement were noted during or after the course of treatment. For the following three months he was nursed at home. He remained emotionally placid, and took a lively interest in people and books, but became virtually anarthric and could barely walk. His intention tremor was so gross that he had to be fed.

March, 1954. In March, 1954, he became once more excitable and hostile to friendly restraining influences, and was readmitted to hospital for a short period before transfer to a mental institution.

April, 1954.—In April he was readmitted to hospital in a semi-comatose condition. He was febrile (no obvious source of infection was detected) and jaundiced. Dependent œdema and ascites were present. He regained consciousness quite suddenly without active treatment. Hypertonicity had increased to the extent of causing virtual immobility. His nocturnal excitement required sedation, and his tolerance for barbiturates, paraldehyde and latterly morphine was remarkable in view of the advanced state of malnourishment and hepatic decompensation at which he had by this time arrived. He lapsed once more into coma and died on May 8, 1954.

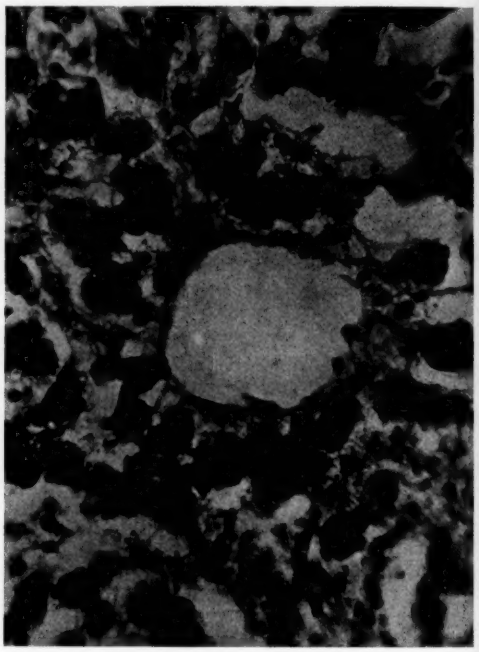

FIGURE II

Histochemical demonstration (rubeanic acid) of abundant copper-containing pigment present in the polygonal cells of the cirrhotic liver in Wilson's disease. (×450)

Autopsy Findings

The body was examined two hours after death. The heart and blood vessels were normal. Hypostatic congestion of the lower lobes of both lungs was present. The spleen was greatly enlarged, weighing 1200 grammes. It was firm and plum-coloured; at the lower pole on the lateral surface there was a recent infarct two centimetres in diameter. The liver weighed 900 grammes. It was brownish in colour, the surface having a sheen like burnished copper. It was coarsely and irregularly nodular, the largest nodules having a diameter of one centimetre (see Figure I). The pancreas was normal.

No œsophageal varices were present, but between the peritoneal layers of the lesser omentum near the cardia there were a number of large tortuous dilated veins, up to 1·5 centimetres in diameter. Similar though smaller vessels were present in the lienorenal ligament and adjacent retroperitoneal tissues. There was also a large tortuous vein (0·75 centimetre in diameter) to be seen on the free edge of the falciform ligament extending from the liver to the umbilicus, where it anastomosed with dilated hypogastric vessels.

The brain weighed 1200 grammes. No obvious macroscopic abnormality was present. The basal ganglia were not appreciably shrunken when sections were examined.

FIGURE III

Deposits of hæmosiderin present in the liver in Wilson's disease. (Stained with Prussian blue reagent, counterstained with eosin. ×200)

Microscopically no abnormality was seen in the adrenals, pancreas, thyroid or pituitary. Active spermatogenesis was absent in the seminiferous tubules, and hyperplasia of the ductal and connective tissue of the breast was seen. In the myocardium and kidneys no abnormal changes were found. The pigment of the skin was melanin present in excess in the cells of the *stratum germinativum*.

In the spleen congestive changes only were found. In the liver the changes of advanced cirrhosis were present. Pseudo-lobules of varying size were interspersed between broad bands of fibrous tissue containing many small bile ducts. There was evidence of much recent necrosis of parenchymal cells. Most of the polygonal cells contained greenish-brown pigment, some of which was thought to be bile pigment and lipofuscin. In addition it was found that some of the pigment gave a positive Prussian blue reaction. The distribution of this hæmosiderin was patchy. The majority of the polygonal cells contained material which gave a strongly positive staining reaction for copper with rubeanic acid, after pretreatment with hydrogen peroxide (modification of the method of Okamoto and Utamura, suggested by Gomori, 1952). Both hæmosiderin and copper-containing pigment were confined to the polygonal cells (see Figures II and III). The microscopic changes in the basal ganglia were not striking. Neurons were reduced in number; in some early degenerative changes were present, but no neuronophagia was seen. Cystic changes were absent, though there was some diffuse increase in glial fibres.

Results of Special Studies

Urinary Copper Excretion.—The patient excreted 50 to 100 times the normal amount of copper in the urine (mean, 1150 microgrammes; range, 640 to 1490 microgrammes in twenty-four hours; 14 and 22 microgrammes in two controls). Values obtained by other investigations are shown in Table II.

TABLE II

Urinary Copper Excretion in Wilson's Disease

Authors	Copper (Microgrammes) Excreted in Twenty-four Hours
Present authors	1490, 1420 (mean of 13 days) 1060, 630
Bearn and Kunkel (1954), 16 cases	703±318
Sullivan, Martin and McDowell (1953), two cases	318, 438
Porter (1949), four cases	207 to 716
Uzman (1953)	17 to 640

Copper Balance.—The copper balance study was divided into two periods, eight days and five days, which made possible the collection of pooled samples of urine, fæces, solid food, milk mixtures and drinking fluids. The results are shown in Table III. Over

TABLE III

Result of Thirteen-Day Copper Balance in a Case of Wilson's Disease[1]

Period of Study	Copper Content (Milligrammes)			Balance
	Food	Fæcss	Urine	
Period I (8 days)	31·4	16·2	9·1	+6·1
Period II (5 days)	22·8	10·7	8·7	+3·4

[1] Mean daily copper intake, 4·2 milligrammes; mean daily fæcal excretion, 2·1 milligrammes; mean daily urinary excretion, 1·4 milligrammes; mean daily copper balance, +0·7 milligrammes.

the period of observation approximately 50% of copper in the diet was absorbed by the gut. Despite a daily urinary copper excretion of approximately 1·4 milligrammes the patient was in positive daily copper balance of 0·8 milligramme (800 microgrammes).

Effect of BAL on Urinary Copper Excretion.—During the balance study the mean daily excretion of copper in the urine was 1400 microgrammes. This study was followed immediately by the first course of BAL, 400 milligrammes being given daily for ten days. The total urinary copper excretion was estimated on the sixth and tenth (last) days of the course. The

amounts excreted, 1610 and 1045 microgrammes, were not greatly in excess of those observed under basal conditions. The hourly excretion of copper in urine after the administration of BAL was observed on the first day of the second course. The total amount of copper excreted on the previous day was 1060 microgrammes (0·66 microgrammes per millilitre). The bladder was emptied at 6 a.m. and two millilitres of BAL were given. Urine was collected thereafter at hourly intervals for seven hours. The increment of copper in the urine appeared within the first hour (4·56 microgrammes per millilitre) and thereafter the excretion of copper fell sharply. A total increment of 550 microgrammes of copper over the previous twenty-four-hour excretion was noted (Figure IV).

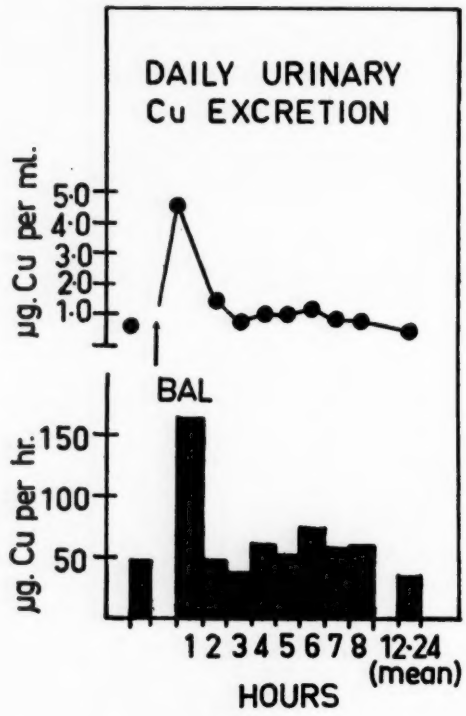

FIGURE IV

Hourly excretion of copper in urine after administration of 200 milligrammes of BAL

Effect of BAL on Blood Copper Levels.—Blood levels of copper were estimated one hour and twelve hours after the administration of BAL on the fifth and tenth days of the first course. On the first occasion a sharp rise was noted from 62 to 93 microgrammes per 100 millilitres, but no significant change was observed on the tenth day (56 to 48 microgrammes per 100 millilitre).

Urine and Blood Copper Content during Calcium ("Versene") Treatment.—The urinary copper output was studied on the first, third and sixth days of the course of "Versene". The increment of copper excretion was small (630 microgrammes rising to 930 and 890 microgrammes). It is not known why at this stage of the patient's illness he had a lower baseline of daily copper excretion.

Blood levels of copper were estimated on the first day at nil, one and two hours after "Versene" had been given, and on the sixth day at nil and twelve hours. No significant change was noted (first day, 57, 58 and 60 microgrammes per 100 millilitres; sixth day, 60 and 80 microgrammes per 100 millilitres).

Copper Content of Organs.—At autopsy a portion of the liver, the spleen, the cerebellum and the right basal ganglia, and a sample of bile were taken for copper analysis. The values obtained, along with those given by other authors, are shown in Table IV.

DISCUSSION

This case, which in all respects conformed to the classical clinical descriptions given of hepatolenticular degeneration, was interesting in that symptoms and signs of hepatic insufficiency—jaundice and œdema—preceded by many years the typical neurological manifestations. In older literature it has been stated that cirrhosis in Wilson's disease is often difficult to diagnose; but in two-thirds of a total of 30 cases studied by Sweet *et alii* (1941), by Herz and Drew (1950), by Homburger and Kozol (1946) and by Franklin and Bauman (1953), one or more abnormalities were revealed by biochemical tests of liver function. Of

TABLE IV

Copper Content of Organs and Bile in Wilson's Disease

Authors	Copper Content (Milligrammes per 100 Grammes Dry Weight)						Copper Content of Bile (Milligrammes per Litre)
	Cortical White Matter	Cerebellum	Caudate Nucleus	Putamen and *Globus Pallidus*	Liver	Spleen	
Cummings (1948): normal figures	1·1 to 8·2	?	3·4 to 9·4	6·1 to 18·8	3·7 to 17·2	?	0·3 to 2·0
Present authors	—	24·2	25·2	31·6	49·0	1·1	1·3
Cummings (1952):							
First case	10·9	—	10·1	8·4	156·4	—	—
Second case	14·7	—	31·8	23·0	55·0	—	—
Third case	12·9	—	13·8	39·9	39·4	—	—
Spillane *et alii* (1952) ..	18	—	27	17 to 37	33 to 37	1	—

the 11 patients studied by Franklin and Bauman, six died in liver failure.

The high level of copper excretion in the urine, the aminoaciduria, and the low blood copper level were also pathognomonic biochemical findings. The normal blood level of copper is about 100 microgrammes per 100 millilitres (range, 69 to 117 microgrammes—Lahey *et alii*, 1953). The mean of several estimations in this case was 68 microgrammes per 100 millilitres. The range of serum copper levels in the 16 cases of Wilson's disease studied by Bearn and Kunkel (1954) was $60 \pm 15 \cdot 4$ microgrammes per 100 millilitres (controls, $108 \pm 9 \cdot 8$ microgrammes per 100 millilitres. (Serum copper levels are approximately 10% greater than whole blood levels.) Copper in serum is present in the form of a metalloprotein, cæruloplasmin (Gubler *et alii*, 1953). This copper-protein has been shown to have oxidase activity (Holmberg and Laurell, 1951) and Bearn and Kunkel (1952, 1954) have shown that "copper enzyme activity" is greatly diminished in Wilson's disease.

The range of copper excretion in urine in this case was 1060 to 1500 microgrammes in twenty-four hours. This was higher than in most cases reported by other authors. The amount of α-amino nitrogen in the urine was increased in comparison with controls, but did not reach the high levels observed by others (Uzman and Denny Brown, 1948; Porter, 1951). The degenerative changes in the liver and basal ganglia in Wilson's disease cannot be directly related to the aminoaciduria, for this seems to be the most variable of the biochemical anomalies; at least two authentic cases of the disease without aminoaciduria have been recorded (Stein *et alii*, 1954; Cooper *et alii*, 1950), and on the other hand siblings of patients with Wilson's disease have been shown to have aminoaciduria without developing clinical manifestations of the disorder. It was suggested by Matthews *et alii* (1952), who showed that copper excretion was increased after the ingestion of large amounts of alanine and glycine, that copper was excreted in the form of amino acid-copper chelates; but this has not been confirmed by subsequent investigators. This perplexing paradox concerning the relation of cupruria and aminoaciduria appears to have been solved by Uzman (1953), who noted that the urine of patients with Wilson's disease contained abnormal oligopeptides with terminal dicarboxylic acids. Chromatographic spots of these oligopeptides reacted strongly with the sensitive copper reagent rubeanic acid. It is believed by Uzman that copper is excreted as a peptide-chelate, and that competition for the tubular resorption of amino acids and this chelate occurs. The finding in this case of an inordinately high level of cupruria with only moderate aminoaciduria would seem to support Uzman's observations.

The balance study performed on this patient appears to confirm the tacit assumption that has been made that in Wilson's disease increased alimentary absorption of copper is taking place. Nearly 50% of the total dietary copper was absorbed, and despite the pronounced cupruria the patient was in considerable positive copper balance. It is fully realized that longer balance studies on controls and on patients with Wilson's disease are desirable, for there is a paucity of information on this aspect of copper metabolism. Nevertheless, the situation is indeed truly analogous to the absorption of iron in relation to the genesis of hæmochromatosis, in which a much higher proportion of the iron "load" presented to the duodenal mucosa is absorbed than by normal patients. It is possible that the same chelating peptide with an avidity for copper present in urine in Wilson's disease is present also in intestinal mucosal cells, as well as in tissues where copper is deposited in excessive amounts.

The results of treatment with BAL have been variable. Cumings (1952) and Schechter and Jones (1953) did not meet with the success that favoured Denny Brown and Porter (1951), and the present patient failed to show any clinical improvement. It is of probable significance that Denny Brown's and Porter's cases were of the more chronic "pseudosclerotic" variety of hepatolenticular degeneration. Perhaps in such cases the daily positive copper balance is less considerable than in the present case, in which there was evidence that neither BAL nor "Versene" was able to put the patient in negative balance. Moreover, the curve of urinary excretion and blood copper levels following BAL administration seems to indicate that only a small amount of copper is available for binding with thiol groups, the bulk being probably more firmly bound to peptide linkages. The continued increase in copper excretion attending the use of "Versene" suggests that it may be a more efficient copper-binding agent; but its avidity for other metals renders it of limited therapeutic value when use over long periods of time is to be considered.

The limitation of alimentary copper intake by modifications of the diet is difficult to effect. Copper is of ubiquitous distribution in food (McCance and Widdowson, 1946), and the problem is complicated by the necessity for a high intake of protein in the presence of impaired liver function. The protein supplements given

to our patient accounted for his comparatively high daily intake of copper (four milligrammes); yet when he did not receive this extra source of nourishment, progressive deterioration of hepatic function occurred.

On the evidence presented it is doubtful whether BAL and "Versene" can be expected to modify the natural history of severe hepatolenticular degeneration. The importance of Uzman's discovery of copper-chelating peptides is to turn attention away from the minutiæ of copper metabolism towards the fundamental disorder of body protein metabolism which presumably accounts for all the biochemical abnormalities in Wilson's disease. The full understanding of abnormal protein metabolism may explain why these patients develop cirrhosis and degeneration of the lenticular nuclei.

SUMMARY

A case of Wilson's disease in a man, aged twenty-two years, with tremor, rigidity, Kayser-Fleischer rings and signs of hepatic insufficiency, is described. The patient died in hepatic coma six years after the onset of his first symptom, jaundice.

Signs of gross portal hypertension were present at autopsy. Examination of the liver revealed severe atrophic cirrhosis, and histochemical study demonstrated the presence of excessive amounts of copper-containing pigment. Chronic degenerative changes were seen microscopically in the lenticular nuclei.

General aminoaciduria of moderate degree was present. Blood copper levels were low, and urinary copper excretion was approximately fifty times normal.

A thirteen-day balance study showed that on a daily dietary intake of four milligrammes, 50% of the copper was being absorbed, and that the patient was in a daily positive copper balance of 0·7 to 0·8 milligramme.

After the administration of BAL there was a rapid rise in urinary copper excretion, which was not sustained; this indicates that much of the copper stored is not available for combination with the –SH groups of BAL. The increase of urinary copper excretion was sustained for longer periods with "Versene". It is improbable that either substance promoted the excretion of copper in greater amounts than were normally absorbed each day in the alimentary tract.

ACKNOWLEDGEMENTS

We wish to acknowledge our indebtedness to Dr. Gerald Moss, who referred the patient for investigation; to Dr. W. S. Davidson for supplies of "Versene"; and to Mr. R. Van Raalte, who prepared the photomicrographs. The work was subsidized by a grant from the Government of Western Australia.

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ADDENDUM

Since this paper was submitted for publication a further case of Wilson's disease has been studied. The patient was a girl, aged ten years, weighing 22 kilograms, who presented with choreo-athetoid movements of all four limbs seven months prior to her death. These neurological manifestations were rapidly progressive; four months later she had become severely dysarthric, and plastic rigidity of the limbs was present. Both the liver and spleen were enlarged on clinical examination, although liver function tests gave results normal in all respects. After a severe melæna she developed ascites. Balance studies were not performed owing to urinary and fæcal incontinence. She was excreting 400 microgrammes of copper daily (150 microgrammes per litre of urine). A ten-day course of BAL caused no really significant clinical improvement. In the middle of the course of BAL the daily urinary copper excretion rose to about 1900 microgrammes *per diem* (670 microgrammes per litre). Filter-paper chromatograms showed a generalized aminoaciduria which was not investigated in detail. The patient died a week after the termination of the course of BAL therapy. Cirrhosis of the liver with considerable fatty infiltration, splenomegaly, œsophageal varices and gliosis of the basal ganglia were noted at the autopsy. The liver contained approximately 80 milligrammes of copper per 100 grammes and the brain 24 milligrammes per 100 grammes.

The striking clinical features of the case were the rapidity of development of neurological signs and symptoms and the lack of immediate response to treatment with BAL, despite an apparent fourfold increase in urinary excretion of copper.

NODULAR GLOMERULOSCLEROSIS

A STUDY OF ITS INCIDENCE, MORPHOLOGY AND ASSOCIATED RENAL LESIONS IN DIABETES MELLITUS[1]

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Kimmelstiel and Wilson (1936) first aroused interest in intercapillary glomerulosclerosis when they noticed in the renal glomeruli of diabetic patients a nodular lesion in which hyaline masses appeared to lie between the glomerular loops. Besides glomerulosclerosis, their eight patients showed widespread nephrotic œdema, hypertension and albuminuria. Although it was soon realized that œdema, hypoproteinæmia, albuminuria and a raised blood cholesterol level did not always accompany intercapillary glomerulosclerosis (Anson, 1938; Simon, 1940; Allen, 1941), Siegal and Allen (1941) and Herbut (1941) showed that the lesion was valuable in the post-mortem diagnosis of *diabetes mellitus*.

The position of the hyaline masses within the glomerulus and their pathogenesis have been much debated since the original description. Allen (1941) disagreed with the intercapillary position and suggested that the nodular masses were the result of thickening of the capillary basement membrane and therefore were intramural in position. Bell (1950 and 1953) also subscribed to this interpretation. However, McManus (1950), using histo-chemical techniques, and Churg and Grisham (1953), after carrying out phase microscope studies, supported the original contention of Kimmelstiel and Wilson. Recently Anderson (1954) suggested that nodular glomerulosclerosis might result from the plugging of glomerular capillaries by a coagulum derived from the blood.

The experimental approach has yielded little information on the pathogenesis of the condition. Lukens and Dohan (1946) noted hyaline deposits in the glomeruli of a dog made permanently diabetic for five years, but their illustrations seem to show a diffuse thickening of the glomerular tuft rather than nodular lesions. Beveridge and Johnson (1950), and Foglia, Mancini and Cardeza (1950), produced diffuse glomerulosclerosis in rats, the former inducing diabetes by alloxan and the latter by pancreatectomy. Mann, Goddard and Adams (1951) also found hyaline masses in the glomeruli of rats rendered diabetic with alloxan, but the lesion was frequently complicated by pyelonephritis and did not stain with collagen techniques. Becker (1952) reported perhaps the nearest experimental analogy to date when he produced retinal aneurysms and glomerular nodules in rabbits made diabetic with alloxan and injected with adrenocorticotropic hormone. His illustration appears a convincing parallel to nodular glomerulosclerosis, and further studies are awaited with interest.

The recorded incidence of intercapillary glomerulosclerosis in diabetic patients has differed enormously, varying from 17% (Warren and Le Compte, 1952) to 82% (Zins, 1949), depending on the individual conception of the lesion. To explain this discrepancy, Bell (1950) suggested that two types of glomerulosclerosis occurred in the kidneys of diabetic patients—namely, the nodular type of Kimmelstiel and Wilson and the diffuse type described by Laipply, Eitzen and Dutra (1944). Many reports indicating a relatively high incidence of intercapillary glomerulosclerosis contained examples of diffuse as well as nodular lesions.

Although excellent articles have appeared recently in Great Britain and the United States (Gauld, Stalker, and Lyall, 1948; Gilliland, 1951; Hall, 1952*a*, 1952*b*; Rogers and Robbins, 1952; Rogers, Robbins and Jeghers, 1952; Robbins, Rogers and Wollenman, 1952), few reports are to be found in the Australian literature. Duffy (1951) examined the incidence of Kimmelstiel-Wilson lesions in the London Hospital autopsies from 1938 to 1948 and found the lesion in five of the 45 diabetic patients on whom autopsies were performed. He also determined the incidence of renal complications in *diabetes mellitus* by surveying 820 patients who attended the Alfred Hospital, Melbourne, during 1950. Twenty-two were considered to have renal complications due to *diabetes mellitus*, based on the presence of

[1] Received on August 10, 1954.

albuminuria, the absence of a history of nephritis and hæmaturia, and the results of renal function tests. Confirmation of the diagnosis of intercapillary glomerulosclerosis was obtained by autopsy in three instances.

The present investigation is an attempt to determine the incidence of nodular glomerulosclerosis in diabetic patients from a large Australian hospital, and to study some factors of possible importance in its production. At the same time, the cause of death and the incidence of associated renal disease in a small series of diabetic patients have been studied. Kidneys which showed diffuse glomerulosclerosis alone were separated from those with nodular glomerulosclerosis, because of the difficulty found in distinguishing diffuse sclerosis resulting from hypertensive arteriolar sclerosis.

MATERIAL AND METHODS

The kidneys used in the study were obtained from the autopsy room of the Royal Adelaide Hospital in the eleven-year period from 1941 to 1951. During this time post-mortem examinations were performed on 170 bodies of adult patients with *diabetes mellitus*. Because of deficiencies in the clinical records, autopsy notes and material available for histological examination, only 104 of these patients were considered suitable for the requirements of the investigation. To serve in some way as a control series, histological preparations of the kidneys of 100 consecutive patients without a history of *diabetes mellitus* were examined.

Prior to 1949, the kidney material was fixed in Zenker's solution; after that year, formaldehyde solution (4%) or Susa fixative was used. Dehydration by means of graded solutions of ethyl alcohol, impregnation with paraffin wax, and staining with hæmatoxylin and eosin were performed. Many sections were stained by the periodic acid-Schiff technique (McManus, 1948) to delineate the glomerular basement membrane, and limited use was made of the Mallory connective tissue stain modified by Kimmelstiel and Wilson (1936), and of the Heidenhain "Azan" technique (Cowdry, 1943). The presence of glycogen in the renal tubules was determined by Best's carmine stain (Carleton, 1926) and the periodic acid-Schiff method (McManus, 1948). No special precautions were taken to retain glycogen in the tissues; but control slides incubated with saliva for fifteen minutes at 37° C. were used in each case.

Nodular glomerulosclerosis was considered to be present in the kidneys if one or more oval to round hyaline masses appeared in the glomerular tufts some distance from the glomerular hilus. The diameter of the nodules selected to distinguish the nodular from the diffuse variety of glomerulosclerosis was approximately 30 μ. This figure had to be arbitrary because of the progressive size of the lesions, but as nodules of this magnitude were readily identified, it served the purpose of separating the two groups.

THE INCIDENCE OF NODULAR GLOMERULAR SCLEROSIS

The lesion occurred in 26 of the 104 patients in the series, an incidence of 25%.

The occurrence of intercapillary glomerulosclerosis recorded by other authors is shown in Table I. Some of the higher percentile figures included diffuse lesions and are therefore not comparable with the present series (Laipply, Eitzen and Dutra, 1944; Goodof, 1945; Zins, 1949). However, it is noteworthy that the incidence of nodular glomerulosclerosis reported by Henderson, Sprague and Wagener (1947) is somewhat lower than most series, despite the inclusion of "early" lesions. Authors who restricted intercapillary glomerulosclerosis to the focal nodular lesion have found an incidence similar to that occurring in the present series (Siegal and Allen, 33%; Bell, 23.6%; Harman, 29.3%; Robbins *et alii*, 26%; Hall, 37.5%). Horn and Smetana (1942) did not accurately define their interpretation of intercapillary glomerulosclerosis, and in view of the high incidence in patients with arteriolar nephrosclerosis (25.4%) an incidence of 22.9% in patients with *diabetes mellitus* suggests the inclusion of diffuse lesions.

TABLE I

Incidence of Intercapillary Glomerulosclerosis in Diabetic Kidneys

Authority	Percentage Incidence	Approximate Female to Male Ratio
Allen (1941)	33·0	—
Bell (1950)	23·6	1·7 : 1
Goodof (1945)	44·0[1]	1·4 : 1
Hall (1952)	37·5	1·4 : 1
Harman (1950)	29·3	—
Henderson *et alii* (1947)	19·5[1]	1·75 : 1
Herbut (1941)	—	2·0 : 1
Horn and Smetana (1942)	22·9	3·8 : 1
Laipply *et alii* (1944)	63·7[1]	1·2 : 1
Robbins *et alii* (1952)	26·0	Slight female preponderance
Warren (1952)	17·0[2]	—
Zins (1949)	82·0[1]	1·2 : 1
Present series	25·0	2·5 : 1

[1] Includes diffuse (fibrotic) glomerular lesions.
[2] An approximate figure only.

By the inclusion of diffuse lesions, the incidence of intercapillary glomerulosclerosis in diabetic patients can be increased by about 50% (Bell, 1953). There were 13 examples in the present series of "definite" diffuse glomerulosclerosis, which, together with the number of subjects with nodular glomerulosclerosis, bring the incidence of intercapillary glomerulosclerosis to 37.5%. However, there is some doubt whether the diffuse lesion is merely an early stage of the nodular type, and while the doubt remains the separation appears justified.

TABLE II

The Age Incidence

Age (Years)	Number of Cases of Diabetes without Nodular Glomerulosclerosis.	Number of Cases of Diabetes with Nodular Glomerulosclerosis.
10 to 19	2 (2·6%)	0
20 to 29	7 (8·9%)	0
30 to 39	4 (5·1%)	0
40 to 49	3 (3·8%)	1 (4%)
50 to 59	24 (30·8%)	4 (16%)
60 to 69	29 (37·2%)	14 (56%)
70 to 79	9 (11·5%)	6 (24%)
Total ..	78	25[1] (100%)
Average age in years	56·1	64·5

[1] The age of one patient with the nodular glomerulosclerosis lesion is not known.

Sex Incidence

Twenty-one of the 66 females in the series (32%) had nodular glomerulosclerosis, and five (13%) of the 38 males were affected.

Previous series have shown a predominance of the lesion in females varying from a slight difference (1.2 : 1.0 in the series of Laipply, Eitzen and Dutra, 1944) to a pronounced one (3.8 : 1.0 in the series of Horn and Smetana, 1942), with an average of 1.8 : 1.0. The sex difference cannot be wholly explained by the greater occurrence of *diabetes mellitus* in females aged over thirty-five years (Joslin *et alii*, 1948), or by the greater frequency of *diabetes mellitus* in females in a large autopsy series (Bell, 1950). This might account for the greater number of females with intercapillary glomerulosclerosis, but not for the greater percentile incidence. Bell (1950) noted that renal arteriolosclerosis was more severe in females than in male diabetic patients, and attributed the higher incidence of glomerulosclerosis to this factor.

Age Incidence

Table II records the age at death in decades of the patients in the series. The greatest number of subjects found to have nodular glomerulosclerosis at autopsy (14) occurred in the sixth decade, with a similar proportion in the seventh. Laipply, Eitzen and Dutra (1944), Robbins *et alii* (1952), and Hall (1952*b*) found the greatest number in the sixth decade; but in Hall's series the largest percentage occurred in the seventh decade.

The average age at death in the group with nodular glomerulosclerosis was sixty-four years, which did not differ greatly from the group without this lesion. The youngest patient with nodular lesions died at the age of forty years, but examples of intercapillary glomerulosclerosis occurring in the second and third decades are recorded (Laipply, Eitzen and Dutra, 1944; Goodof, 1945; Henderson, Sprague and Wagener, 1947; Millard and Root, 1948; Mann, Gardner and Root, 1949; Alwall, Ekelund and Oras, 1950; Glushien, Reiter and Fischer, 1952). Wilson, Root and Marble (1951) also drew attention to the importance of renal vascular disease in young diabetic patients.

ASSOCIATED RENAL DISEASE

Associated renal disease, excluding incidental findings such as cortical adenomata, medullary fibromata, and malignant and leuchæmic deposits, are shown in Table III.

The incidence of glycogen infiltration in the renal tubular cells was 12%. Warren and Le Compte (1952) suggested that glycogen

TABLE III

Associated Renal Lesions in 104 Patients with Diabetes Mellitus

Type of Kidneys	Pyelonephritis		Arteriolosclerosis (Moderate to Severe)	Glycogen Nephrosis	Necrotizing Papillitis
	Acute	Chronic			
Kidneys with nodular glomerular lesions (26)..	4[1]	2	23	1	1
Kidneys without nodular lesions (78).. ..	8[2]	6	21	11	0

[1] One case was accompanied by chronic pyelonephritic lesions.
[2] Two cases were accompanied by chronic pyelonephritic lesions.

occurred in the kidneys of almost all patients with diabetes before the introduction of insulin therapy, and Robbins (1948) found it in 42% of kidneys from patients with diabetes in an autopsy series from 1933 to 1941. Gross examples now appear to be relatively uncommon, occurring half as frequently as nodular glomerulosclerosis in this series. Nodular glomerulosclerosis was associated with glycogen "nephrosis" in only one patient, and McManus (1950) also thought the combination of the two lesions unusual. Auroi (1943), McManus (1950), Zubrod, Eversole and Dana (1951), and Robbins *et alii* (1952), suggested that the lower urinary excretion of glucose and the milder diabetic state in patients with glomerulosclerosis might account for this.

TABLE IV
Relationship of the Kimmelstiel-Wilson Lesion and Renal Arteriosclerosis

Renal Arteriolosclerosis	Number of Patients	Number of Patients with Kimmelstiel-Wilson Lesion	Kimmelstiel-Wilson Lesion Percentage (Approximate)
Nil to slight ..	22	1	4·5
Mild	38	2	5·3
Moderate ..	19	8	42·0
Severe	25	15	60·0

The association of nodular glomerulosclerosis and renal arteriosclerosis has been repeatedly stressed (Kimmelstiel and Wilson, 1936; Horn and Smetana, 1942; Laipply, Eitzen and Dutra, 1944; Harman, 1950; Bell, 1950; Allen, 1951; Robbins *et alii*, 1952; Hall, 1952*b*). In this series there was a pronounced increase in the frequency of the Kimmelstiel-Wilson lesion as the arteriolar disease increased in severity (Table IV). Newburger and Peters (1939) suggested that the pathogenesis of intercapillary glomerulosclerosis appeared to depend on severe and extensive arterial and arteriolar degeneration, but there is evidence to suggest that this is not strictly correct. One patient in this series with nodular glomerulosclerosis showed no arteriolar disease, and two others had minimal arteriolar lesions. Previous authors have also recorded cases in which renal arteriolosclerosis was absent or slight (Laipply, Eitzen and Dutra, 1944; Henderson, Sprague and Wagener, 1947; Hall, 1952*b*). Hyaline sclerosis of the efferent arterioles of the glomeruli was commonly associated with nodular glomerulosclerosis, occurring in 23 of the 26 patients. The hyaline material stained similarly to that in the afferent arterioles, but in every instance was less extensive. The sections from two patients showed only slight efferent sclerosis, which in another appeared to be absent. Allen (1941) considered that the nodular lesion might occur in glomeruli with normal arterioles, and conversely, might be absent from glomeruli with thickened efferent arterioles; in this series several examples of efferent arteriolarsclerosis occurred in kidneys without nodular lesions. Allen stated that the efferent arterioles were apparently normal in non-diabetic hypertensive kidneys, and Harman (1950) confirmed the absence of efferent arteriolar disease in 90 non-diabetic patients with renal arteriolonephrosclerosis, and in 100 normal controls. However, this does not exclude the possibility of the lesion occurring in non-diabetic patients, and larger series need to be studied to confirm this hypothesis.

DURATION OF DIABETES MELLITUS

The greatest number of deaths among patients with nodular glomerulosclerosis occurs in the sixth and seventh decades of life, which suggests that the duration of diabetes may be of importance in determining the appearance of intercapillary glomerulosclerosis (Hall, 1952*b*).

TABLE V
Duration of Diabetes Mellitus and Nodular Glomerulosclerosis

Known Duration (Years)	Number of Patients	Number with Nodular Glomerulosclerosis
0 to 5	23	6 (25%)
6 to 10	16	4 (25%)
11 to 15	8	4 (50%)
16 to 30	6	3 (50%)

The known duration of the disease (from onset of symptoms to death) was established in approximately one-half of the series (Table V). and in 17 of the 26 patients with the lesion it varied from eighteen months to thirty years.

Other authors have suggested a relationship between the duration of diabetes and the occurrence of the Kimmelstiel-Wilson lesion (Goodof, 1945; Henderson, Sprague and Wagener, 1947; Colwell, 1948; Hall, 1952*b*). However, Laipply, Eitzen and Dutra (1944) and Rifkin, Parker, Polin, Berkman and Spiro (1948) could find no definite correlation. The presence in some patients of gross nodular lesions with a short duration of diabetes may possibly be explained by the inexact knowledge of the time of onset of the disease (Henderson, Sprague and Wagener, 1947). However, a long

duration of diabetes does not necessarily lead to the development of nodular glomerulosclerosis, as one patient in this series who had diabetes for thirty years showed no lesions. The seven patients in this series, and the four quoted by Hall (1952*b*) without nodular glomerulosclerosis after more than ten years of diabetes, suggest that duration is not the most important factor in the development of nodular lesions.

Œdema and Albuminuria in Relationship to the Kimmelstiel-Wilson Lesion

Kimmelstiel and Wilson considered that œdema was one of the characteristic clinical features of patients with intercapillary glomerulosclerosis. However, in this survey œdema occurred in only four of the 26 patients, and only one of these appeared to have œdema of the nephrotic variety. In the remaining three patients the œdema could be explained on non-renal grounds.

The recorded incidence of the nephrotic syndrome in patients with intercapillary glomerulosclerosis is variable. Newburger and Peters (1939), Simon (1940), and Porter and Walker (1941) suggested a high occurrence of "nephrosis", and Siegal and Allen (1941) noted 11 patients with the nephrotic syndrome in 105 diabetics. In the series of Anson (1938), of Laipply, Eitzen and Dutra (1944), of Goodof (1945), of Henderson, Sprague and Wagener (1947), and of Bell (1950), nephrotic œdema occurred infrequently, and Kimmelstiel and Porter (1948) stated that it appeared in less than 10% of patients with intercapillary glomerulosclerosis. It has been suggested that the nephrotic syndrome in diabetes is always accompanied by intercapillary glomerulosclerosis (Kimmelstiel and Porter, 1948); but Lefeber and Decherd (1946) described two patients with *diabetes mellitus*, proteinuria, hypoproteinæmia and generalized œdema whose kidneys did not contain the Kimmelstiel-Wilson lesion. Rogers and his colleagues (1952) found a greater incidence of œdema in diabetic patients with the lesion than in those without it, but suggested cardiac failure rather than renal disease as the factor causing the difference. Lefeber and Decherd (1946), Henderson, Sprague and Wagener (1947), and Rifkin *et alii* (1948) agreed that the presence of œdema was related in the majority of cases to cardiac failure.

The results of urine examinations were recorded for 23 of the present patients with nodular glomerulosclerosis, and 16 showed albuminuria at some stage during the course of the diabetes. In seven cases the albuminuria was noted for periods varying from six months to five years, but in the remaining nine it was found in the terminal stage of life associated with coma or myocardial infarction.

Twenty of 60 patients without nodular glomerulosclerosis in the present series for whom the results of urine examinations were recorded showed albuminuria, an incidence of 33 per cent. Laipply, Eitzen and Dutra (1944) and Henderson, Sprague and Wagener (1947) found albuminuria in 71% and 76% of diabetic patients without glomerulosclerosis, and Harman (1950) noted an incidence of 35% in his corresponding group. Bell (1950) stated that the majority of diabetic patients showed "some degree of albuminuria", the amount and frequency usually being greater in association with intercapillary glomerulosclerosis. It is apparent that the presence of albuminuria in a patient with *diabetes mellitus* does not alone justify the diagnosis of intercapillary glomerulosclerosis, nor does its absence exclude it.

The Cause of Death

Until recently, little interest had been shown in the cause of death of patients with intercapillary glomerulosclerosis. Henderson, Sprague and Wagener (1947) stated that disease of the cardio-vascular system killed 52% of patients with intercapillary glomerulosclerosis and 30% of those without it. Rogers *et alii* (1952) compared the cause of death in 100 diabetic patients with intercapillary glomerulosclerosis and 287 diabetics without sclerosis, and suggested that certain differences warranted attention. For example, uræmia was nearly twice as common and cardiac failure and cerebrovascular accidents were more common in the group with glomerulosclerosis than in the controls.

Analysis of the present series was hampered by the small numbers, but some differences between the two groups were noted (Table VI). One patient with and 18 without nodular glomerulosclerosis died in diabetic coma, an incidence of 3.8% and 23% in the respective groups. This difference is statistically significant at the 0.5% level. This is of interest in view of the report by Zubrod, Eversole and Dana (1951) that diabetic acidosis is virtually absent in patients with the Kimmelstiel-Wilson lesion, and the absence of diabetic coma from the causes of death of 32 patients described by Henderson, Sprague and Wagener (1947). On the other hand, Rogers *et alii* (1952) found no significant difference in the incidence of diabetic coma in the two groups.

TABLE VI

Causes of Death in the Present Series

Cause of Death	Diabetes with the Kimmelstiel-Wilson Lesion		Diabetes without the Kimmelstiel-Wilson Lesion	
	Number of Patients	Approximate Percentage	Number of Patients	Approximate Percentage
Myocardial infarction	7	27·0	11	14·0
Myocardial failure (excluding infarction)	1	3·8	6	7·7
Pulmonary infection (including tuberculosis)	—	—	4	5·1
Infection (excluding pulmonary causes)	5	19·0	5	6·4
Cerebral hæmorrhage, softening or embolism	2	7·7	11	14·0
Diabetic coma	1	3·8	18	23·0
Renal failure	5	19·0	2	2·6
Unknown or doubtful causes, malignant disease and other causes	5	19·0	21	27·0

Renal failure caused the deaths of five of the 26 patients in the group with nodular glomerulosclerosis but of only two of the 78 patients without it, incidences of 19% and 2.6% respectively. Rifkin *et alii* (1948) reported death in uræmia in nine of a group of 22 patients with the Kimmelstiel-Wilson lesion, and Hall (1952*b*) reported it in five of a group of 45. Rogers *et alii* (1952) also found uræmia to be a more common cause of death in this group than in the controls.

THE MORPHOLOGICAL AND STAINING CHARACTERISTICS OF NODULAR GLOMERULOSCLEROSIS

Unless otherwise stated, the following description applies to the appearances seen in sections stained with hæmatoxylin and eosin.

The earliest nodular lesion is a biconvex or ovoid, homogeneous, eosinophilic structure situated towards the periphery of the glomerular tuft, sometimes appearing paler than the adjacent capillary basement membrane. As it enlarges, the lesion becomes circular in outline and is characterized by the presence of included nuclei occurring in greatest numbers towards the periphery. In the established nodule, faint laminations are often visible with the low-power objective of the microscope, forming concentric arcs of circles which become progressively more crowded towards the periphery of the nodule (Figure I). Reticular silver techniques applied to the lesion stain the central part of the nodule a light grey or brown, while the outer regions show black strands, partly encircling the circumference.

A characteristic accompaniment of the larger nodules is a capillary loop which appears partly or completely to surround it. The loop is either collapsed and difficult to make out, or dilated and filled with red cells. The outer wall of the loop adjacent to Bowman's capsule is thin, and not thickened unless adherent to the latter. In my experience these loops surround established nodules, but do not occur as isolated findings in glomeruli without nodular glomerulosclerosis. However, Anderson (1954) demonstrated a glomerular aneurysm in a tuft not showing nodular glomerulosclerosis.

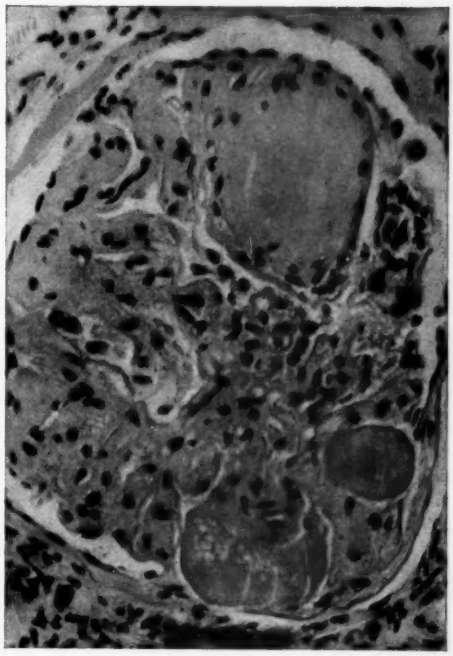

FIGURE I

A glomerulus containing a laminated nodular lesion (above) and two "fibrinoid" lesions (below) (×320)

With the appearance of established nodular lesions (but sometimes in glomeruli showing only diffuse glomerulosclerosis), another change

occasionally occurs. Bright pink material collects between the parietal epithelial cells of Bowman's capsule and the basement membrane, tending to push the cells into the capsular space. The material often assumes an oval or circular shape, is glossy and refractile, and sometimes appears to infiltrate the substance of the thickened Bowman's capsule (the capsular drops of Barrie, Askanazy and Smith, 1952). The material differs in situation, appearance and staining characteristics from the

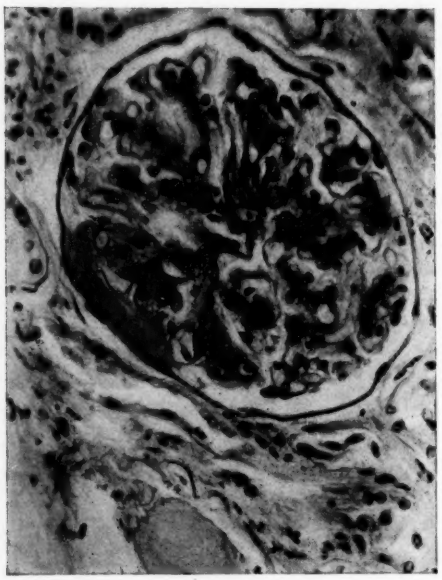

FIGURE II

Hyaline material projecting into Bowman's capsular space and impinging against glomerular loops. (×280)

Kimmelstiel-Wilson nodules, and resembles the hyaline material occurring in the walls of the afferent and efferent arterioles (Figure II).

Five of the patients with nodular glomerulosclerosis showed yet another glomerular lesion, which is seen in hyalinized glomeruli and consists of a globular or crescentic mass with a pink, glossy appearance, containing no laminations, and situated near the periphery of the tuft. This " exudative " material (see also Hall, 1952*a*) is often more obvious than the nodular lesion in areas of kidney undergoing ischæmic atrophy, but both lesions can occur together in the same glomerulus (Figure I). At first it was thought that the material represented a degenerative change in the nodular lesion, until it was noted in glomeruli without nodular glomerulosclerosis. Translucent circular areas appear in the larger masses, which stain poorly or not at all with eosin. The five patients with the exudative lesion died in renal failure with much elevation of the blood urea nitrogen level, and form a group similar to those described by Hall (1952).

Neutral fat rarely occurs in the uncomplicated nodule, but with enlargement of the lesion and ischæmic obliteration of the tuft and capsular space, small globules appear which stain orange with Sudan III. The " exudative " material and the hyaline arteriolar lesions, on the other hand, contain large amounts of sudanophilic material, and doubly refractile lipoid was demonstrated in the former by Hall (1952*a*). Anisotropic lipoid was not detected in typical nodular lesions from two patients in this series.

The tinctorial differences between the " exudative " material, the capsular drops and the arteriolar hyaline material on the one hand, and nodular glomerulosclerosis on the other, can be demonstrated by the use of the Heidenhain " Azan " technique. The former lesions stain bright red in contrast with the deep blue of the laminated nodules. Koss (1952) stressed the morphological and staining differences between the " exudative " material and nodular glomerulosclerosis, the former giving some of the staining reactions of " fibrinoid ".

It was found difficult to localize the fibrinoid material in the tuft by the examination of grossly affected glomeruli, but the study of smaller deposits showed the material to appear first beside the capillary lumen between the endothelial nuclei and the basement membrane (see also Barrie, Askanazy and Smith, 1952). The existence of a potential space between the capillary endothelium of the tuft and the basement membrane had been previously suggested by Jones (1951), and accumulation of the " fibrinoid " in this region tends to close the capillary lumen, producing its characteristic crescentic structure.

THE OCCURRENCE OF NODULAR GLOMERULOSCLEROSIS IN NON-DIABETICS

In accordance with the histological criteria used for the acceptance of nodular glomerulosclerosis in the diabetic patients, 100 control kidneys from patients without a history of *diabetes mellitus* were examined, and no classical nodules were detected.

I have examined kidney sections from approximately 400 post-mortem examinations, and found nodular glomerulosclerosis occurring

only in diabetic patients. This is too small a number from which to draw firm conclusions, but it suggests that for practical purposes nodular glomerulosclerosis is limited to patients with *diabetes mellitus*. In addition, no examples have occurred in non-diabetics at the Memorial Hospital, New York, at the Kingsbridge Veterans' Hospital, or at the Army Institute of Pathology (Allen, 1951).

However, examples of intercapillary glomerulosclerosis occurring in the absence of *diabetes mellitus* have been reported, including four examples of nodular glomerulosclerosis (Bell, 1950; Allen, 1951; Hall, 1952*b*). Nevertheless, the absence of glucose in the urine or a normal blood sugar level does not entirely exclude a previous diabetic state. For example, Rogers *et alii* (1952) reported three instances of intercapillary glomerulosclerosis apparently occurring in non-diabetics, whose past history and hospital records revealed evidence of *diabetes mellitus*.

Other diseases, including amyloidosis and glomerulonephritis, produce nodular hyaline lesions in the renal glomeruli, but these can be distinguished by careful histological examination (see also Allen, 1951; Hall, 1952*b*; Jones, 1953).

So few authentic examples of nodular glomerulosclerosis have occurred in patients without *diabetes mellitus* that its appearance in "non-diabetics" suggests latency of the disease.

SUMMARY

The autopsy incidence of nodular glomerulosclerosis was determined in a series of 104 diabetic patients from the wards of the Royal Adelaide Hospital from 1941 to 1951 inclusively.

The incidence of 25% approximated figures given by previous authors who restricted their study to nodular lesions.

Nodular glomerulosclerosis was twice as common in females as in males.

The greatest proportion of patients with nodular glomerulosclerosis died in the sixth and seventh decades.

Renal arteriolosclerosis, although commonly associated with nodular glomerulosclerosis, was absent in one patient and therefore could not be regarded as the essential causative factor.

The known duration of *diabetes mellitus* in patients with nodular glomerulosclerosis varied greatly, and was not always of the order of ten years. A long history of *diabetes mellitus* was not necessarily associated with the presence of nodular glomerulosclerosis.

Œdema of the nephrotic variety occurred once in the group with nodular glomerulosclerosis. Albuminuria alone did not justify the diagnosis of the lesion, nor did its absence exclude it.

Diabetic coma was an uncommon cause of death among patients with nodular glomerulosclerosis. However, renal failure was more common in patients with nodular lesions than in those without.

Five patients in the series showed an "exudative" or fibrinoid glomerular lesion which morphologically was different from nodular glomerulosclerosis. It was accompanied by much arterial and arteriolar disease of the kidney, and by death from renal failure.

Nodular glomerulosclerosis occurred only in patients with *diabetes mellitus*. Amelioration of the diabetic state might account for the occasional presence of typical nodules in "non-diabetics".

ACKNOWLEDGEMENTS

I am grateful to Professor J. S. Robertson and Dr. R. A. Barter, of the Department of Pathology, University of Adelaide, for advice and encouragement given me during this study. Special thanks are due to Miss Margaret Gladstone for assistance with histological preparations.

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A CLINICAL STUDY OF LEPTOSPIROSIS IN NORTH QUEENSLAND[1]

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THESE studies are based on 115 cases of leptospirosis diagnosed and investigated at the Innisfail Field Station of the Queensland Institute of Medical Research in the period from July, 1952, to December, 1953. They overlap the series whose epidemiology has been reviewed (Derrick *et alii*, 1954); cultures of leptospiræ isolated from 68 of these patients were discussed in a review of 89 strains of North Queensland leptospiræ (Smith *et alii*, 1954).

Clinical data for these patients were obtained from hospital records and from pro-formas filled in by the attending practitioner. In addition almost all in the period from March to December, 1953, were seen by the writer. Complete clinical records were not available in all cases.

CRITERIA OF DIAGNOSIS

Sixty-eight cases were diagnosed by isolation of leptospiræ in blood culture; in all of these agglutinins developed against the strain isolated.

The remainder were diagnosed by agglutination reactions, 44 by examination of paired sera. Three cases are included in which diagnosis was based on the clinical picture and high convalescent titre in the single sample obtained. One of these patients was a child, in whom the possibility of a previous infection would be small.

Diagnosis of serotype presented some difficulties; of the 47 cases diagnosed by serology, in many agglutinins developed against more than one type of leptospira; in seven cases cross-agglutination and anamnestic reactions made the nature of the infecting type obscure. These have been allotted to the group considered most likely.

INCIDENCE OF SEROTYPES

Ten serotypes are represented. They are as follows: *icterohæmorrhagiæ*, three cases; *canicola*, six; *australis A*, 38; *australis B*, 29; "Robinson", six; *pomona*, two; *hyos*, seven; "Kremastos", 13; "Szwajizak", five; "Celledoni", six.

It will be noted that 36 cases were due to serotypes not recognized in Australia before 1951.

AGE INCIDENCE

The youngest patient in the series was aged nine years, the oldest fifty-eight years. The average age of the series was twenty-eight years. The average ages for the various serotypes were as follows: *icterohæmorrhagiæ*, twenty-seven years; *canicola*, thirty-one years; *australis A*, twenty-nine years; *australis B*, twenty-seven years; "Robinson", thirty-one years; *pomona*, twenty-eight years; *hyos*, thirty-one years; "Kremastos", twenty-one years; "Szwajizak", twenty-seven years; "Celledoni", thirty-seven years. (In the larger series of Derrick *et alii*, 1954, the mean age of *australis A* infection was significantly higher than that of *australis B* or "Kremastos".)

ONSET

Leptospirosis is usually described as being of sudden onset. It is of interest that only eight patients were admitted to hospital on the day of onset; 37 were admitted on the following day. The longest duration of illness before admission to hospital was seven days (in two cases).

The average duration of illness before admission to hospital was 2·1 days. Averages for the various types were as follows: *icterohæmorrhagiæ*, 2·3 days; *canicola*, 3·4 days; *australis A*, 1·8 days; *australis B*, 2·2 days; "Robinson", 1·9 days; *pomona*, 1·3 days; *hyos*, 2·0 days; "Kremastos", 2·3 days; "Szwajizak", 2·4 days; "Celledoni", 2·5 days. Some of the differences recorded may be real, but they did not reach the level of statistical significance in the present series.

SYMPTOMS

Symptoms and signs found in this series are summarized in Table I.

Headache was the commonest presenting complaint. Precise localization (for example, occipital, retro-orbital *et cetera*) varied among individual patients. In most cases it was coincident with the febrile period. However,

[1] Received on September 2, 1954.

headache was a prominent feature in cases associated with the "post-leptospiral neurasthenia" mentioned below.

Generalized muscle pain, in back and limbs, was very common, although localization in limb pain—to joints, bone or muscle—was often very indefinite. Pain in the calves, a feature stressed by Kotorii (1935) and Hughes (1953), was of diagnostic value at times in suggesting leptospirosis. However, few patients had the classical picture of "agonizing muscular pain and tenderness" described by Fletcher (1927).

"Shivers", "feverishness" and "chills" were common and were frequent presenting symptoms. Twenty-eight patients had rigors recorded during their course in hospital.

TABLE I

Frequency of Clinical Features Recorded in 115 Cases of Leptospirosis

Clinical Feature	Number of Cases	Clinical Feature	Number of Cases
Headache	93	Lymph gland enlargement ..	64
Limb pains ..	69	Liver tenderness	47
Shivers	61	Conjunctival injection	31
Vomiting	48	Albuminuria ..	23
Backache ..	44	Splenomegaly ..	10
Abdominal pain	34	Neck stiffness ..	9
Sore eyes	31	Rash	5
Cough	22	Suspected jaundice	4
Frequency of micturition ..	5	Epistaxis ..	1
Dysuria	5	Hæmaturia ..	1
Subjective neck stiffness ..	5		

Vomiting was a feature in 48 cases; also nine patients complained of nausea. Thirty-four had abdominal pain, usually epigastric and sometimes very severe. Broom (1953*c*) has written that "vomiting and abdominal pain may be prominent features and suggest an abdominal emergency". In some cases abdominal pain was localized to the right hypochondrium and associated with liver tenderness. Anorexia and constipation were invariable. No patient had diarrhœa, in contrast with some overseas series (for example, six out of 32 in Fletcher's series, 1927).

No serotype correlation could be found in the alimentary symptoms.

Respiratory symptoms were less frequent. Twenty-two patients complained of, or would admit to, a cough on their admission to hospital; two complained of a "cold in the head", one of a hoarse voice. Of the 22 with a cough, six had abnormal physical signs (rales or rhonchi) recorded; one of these had the clinical and X-ray picture of bronchopneumonia; the causative serotype was an *australis B*. It is of interest that Johnson (1950) described a fatal case of *australis A* infection with bronchopneumonia.

Urinary symptoms were uncommon; five patients complained of frequency of micturition and five of dysuria (including three with both), and two of some difficulty in micturition. In each case the urinary symptoms were short-lived.

Eye symptoms were common; 31 patients had sore eyes with photophobia; two noticed blurring of vision (one *canicola* serotype, one *icterohæmorrhagiæ*).

One patient (*australis B* serotype) complained of tinnitus and deafness; two (one "Szwajizak" serotype, one *icterohæmorrhagiæ*) complained of insomnia; five complained of neck stiffness, as distinct from patients showing objective neck stiffness (discussed below).

PHYSICAL SIGNS

No patient in this series had definite proven icterus, although in four cases a doubtful icteric tint was noticed in the eye for a short period. Whether these patients were actually jaundiced is doubtful, as racial pigment and conjunctival injection make the clinical diagnosis of minimal degrees of icterus most difficult. One such patient, considered to have icteric staining in the eyes, was found to have a normal serum bilirubin content at that time. Two patients were found to have biliuria on a single occasion each.

In 47 cases liver tenderness was found, and in 11 of these the liver was considered palpably enlarged.

Tenderness under the left costal margin was found in 19 cases; in 10 of these the spleen was palpable. The degree of enlargement was never great, and in no case did the spleen remain palpable for more than two days. Splenomegaly is a variable feature in recorded series of leptospirosis; thus Rosenberg (1951) describes it in 2·4% of 198 cases of canicola fever, while it was prominent in accounts of Fort Bragg fever (Gochenour *et alii*, 1952).

There was no indication that any one serotype was more likely than others to cause liver tenderness. The 47 cases included two *icterohæmorrhagiæ*, two *canicola*, 13 *australis B*, 13 *australis A*, three "Robinson", one *pomona*, three *hyos*, seven "Kremastos", two "Szwajizak" and one "Celledoni" infections. Splenomegaly, biliuria and jaundice were more common in *australis A* infections, although the numbers are small. The 10 patients with splenomegaly include five with *australis A* infections, one with *australis B*,

one with " Robinson " and three with " Kremastos ". Both cases in which biliuria occurred were due to *australis A*, as were three of the four cases in which the presence of jaundice was questioned.

A rash was seen on five patients; causative serotypes were *australis A* (twice), " Kremastos ", " Szwajizak " and " Celledoni ". The rash appeared on the third day in three, on the fifth day in one; the date of onset in the other was not recorded. In each case it was macular and limited to the trunk, and lasted up to twenty-four hours.

Neck stiffness was recorded in nine cases, in each on the patient's admission to hospital; the duration was not longer than twenty-four hours. The diagnosis of leptospirosis was established on other clinical features, and it was uncommon for neck stiffness to cause any difficulty. Lumbar puncture was performed in only two cases, and each time clear fluid was obtained under normal pressure, and with a cell count of nil. Causative serotypes in these cases were *australis A* (three), " Kremastos " (two) and *australis B*, " Robinson ", *pomona*, " Celledoni " (one each). No patient in this series showed meningeal symptoms at the time of the secondary rise in temperature, and no patient had a lumbar puncture at that time.

Lymph gland enlargement was the commonest abnormality found on physical examination. This is in keeping with previous Australian reports; for example Morrissey (1934) found that enlarged axillary glands were a constant feature. Some overseas observers have not been of that opinion; Van Thiel (1948) reported that " glandular swellings rarely occur ".

Lymphadenopathy was found in 64 cases; in 21 the inguinal glands were involved, in seven the axillary glands, and in 36 both inguinal and axillary glands. Cervical adenitis was found in three, and enlargement of an epitrochlear gland was recorded once.

All 10 serotypes are represented in these 64 cases; there is no suggestion that lymphadenopathy, in degree or site, is correlated with the infecting type.

Albuminuria was found in 23 cases; in all it occurred during the febrile period and subsided before the patient's discharge from hospital. No patient had oliguria (Broom 1953*c*). One patient had hæmaturia and two had transient biliuria.

Serotypes represented in the 23 patients with albuminuria were as follows: *canicola* (two), *australis A* (nine), *australis B* (three), " Robinson " (one), *pomona* (two), *hyos* (three), " Kremastos " (one), and " Szwajizak " (two). However, the differences are not significant.

Injection of conjunctivæ, usually associated with photophobia, was described in 31 cases (one *icterohæmorrhagiæ*, ten *australis A*, ten *australis B*, two *pomona*, two *hyos*, two " Kremastos " and four " Szwajizak " infections). This was the only eye abnormality detected during the acute illness. One patient (infected with *pomona*) had iritis four months after his acute infection.

Only two patients had episodes of bleeding; one (*australis A*) had an epistaxis; another (also *australis A*) had macroscopically evident hæmaturia for forty-eight hours.

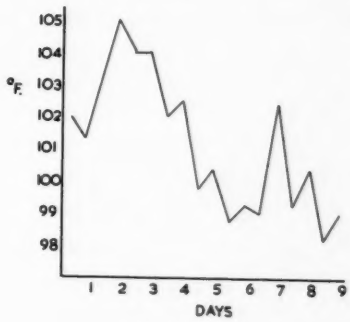

Figure I

Temperature chart in leptospirosis (*australis B*); no antibiotic therapy given

Clinical accounts of leptospirosis usually state that the pulse rate, while increased, is not in proportion to the temperature (for example, Broom 1953*b*). Some observers have ascribed the relative bradycardia to jaundice when it is present (Haunz and Cardy, 1952). In the present series patients with a relatively low pulse rate at the height of fever usually proved to have low resting pulse rates in convalescence; whether that represented their normal pulse rate is, of course, debatable. The majority had temperatures and pulse rates varying in proportion.

Course of Illness

The febrile course of leptospirosis, as shown by the temperature chart, has a fairly characteristic pattern best seen in untreated cases (see Figure I).

The highest temperature recorded was 106·4° F. in an *australis A* infection; 44 patients reached above 104° F., and only eight failed to reach 102° F. during their hospital

course. Four of these eight had been ill four or more days before their admission to hospital.

No correlation could be found between maximum temperature and causative type of organism.

The total duration of fever averaged 5·2 days, with a standard deviation of 1·3 days. This was measured from the onset to the first afebrile day—that is, a twenty-four hour period when the temperature did not exceed 99° F.; a secondary rise in temperature was not included.

The average durations of fever for the individual serotypes are shown in Table II.

TABLE II

Average Duration of Fever Due to Ten Serotypes of Leptospirae

Serotype	Number of Cases	Average Duration of Fever, in Days	Standard Deviation
icterohæmorrhagiæ ..	3	4·6	—
canicola	6	6·5	—
australis A	37	4·5	1·6
australis B	29	5·8	1·5
" Robinson "	6	5·8	—
pomona	2	5·0	—
hyos	7	3·7	—
" Kremastos "	13	5·0	1·3
" Szwajizak "	5	5·8	—
" Celledoni "	6	5·6	—
Whole series ..	114	5·2	1·3

The difference in duration of fever between *australis A* and *australis B* infections is significant. This conclusion does not depend on the figures set out in Table II. In the means quoted there, biases have been introduced by the existence of differential effects due to types of treatment and the different proportions in which these treatments occur. The difference in duration of fever between *australis A* and *australis B* infections is also significant when unbiased estimates are obtained by the method employed in the statistical analysis.

It is of interest to compare these figures with those of Johnson (1950), whose series of patients was treated either without antibiotics or with low dosages. His figures were as follows: (i) *australis A* infections: duration of fever, five to 17 days with an average of 8·2 days (six cases); (ii) *australis B* infections: duration five to 22 days, with an average of 10·9 days (29 cases).

The shorter duration of fever in the present series is striking and will be discussed further under " Treatment ".

A secondary rise in temperature was seen in 41 cases. This was defined as a temperature of over 99° F. after the temperature had been normal for twenty-four hours. Obviously this definition will exclude such cases as those shown in Figure I, in which a secondary rise occurred without an afebrile period. A typical secondary rise is shown in Figure II.

The rise in temperature occurred from the fifth to the fifteenth day; the average day of appearance was the eighth (standard deviation, 1·9). The duration was from one to four days; in 29 cases the rise lasted for only one day, in nine for two days, in one for three days, and in two for four days. The serotypes responsible for these 41 cases were as follows: *icterohæmorrhagiæ* (one), *canicola* (one), *australis A* (16), *australis B* (nine), *pomona* (one), *hyos* (one), " Robinson " (two), " Kremastos " (seven), " Szwajizak " (one) and " Celledoni " (two). The differences in serotype incidence do not reach the level of significance.

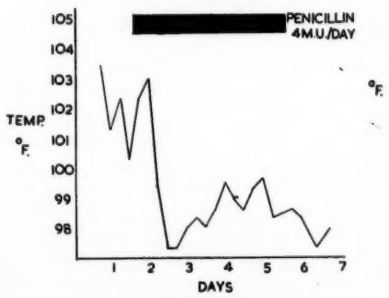

FIGURE II

Temperature chart in leptospirosis (*australis A*); treated with high dosage penicillin and showing pronounced secondary rise

It was uncommon for the secondary rise in temperature to be accompanied by symptoms, although several patients had a return of headache and vomiting.

Many of these patients were followed during a period of up to eighteen months after their illness. A slow convalescence was a common feature, and this was particularly obvious in four cases (one *australis A* infection, one *hyos*, one " Kremastos " and one " Celledoni "). These patients complained of lassitude, vague generalized muscular pains, giddiness and inability to work. Examination revealed some vasomotor instability and palpably enlarged inguinal glands. The condition appeared to subside in three to six months after the illness. A functional element appeared to be present in several of them.

One patient had iritis four months after his attack of *pomona* leptospirosis; beyond conjunctival injection no eye abnormality was seen during his acute illness.

Differential Diagnosis

In the coastal belt of North Queensland leptospirosis must be distinguished from a number of other febrile illnesses. Of these the most important is scrub typhus; "Q" fever occurs in small numbers; there are also short fevers, clinically very similar to the milder cases of leptospirosis, which are not yet fully investigated. While these illnesses have individual characteristics, they can and frequently do present with only non-specific febrile symptoms, and diagnosis on the patient's admission to hospital may be difficult or impossible. It will be noted that a considerable proportion of this series received both penicillin and chloramphenicol because of uncertainty or alteration in clinical diagnosis.

Table III

Comparison of Clinical Features of Leptospirosis and Scrub Typhus

Clinical Features	Percentage Incidence in Leptospirosis	Percentage Incidence in Scrub Typhus
Headache	81	96
Backache	38	16
Limb pain	60	25
Cough	19	25
Frequency of micturition	4	8
Dysuria	4	8
Vomiting	42	8
Abdominal pain	29	21
Rash	4	41
Eschar	Nil	37
Splenomegaly	9	12
Hepatomegaly	10	16
Liver tenderness	41	37
Chest signs	5	12
Lymphadenopathy	56	58
Neck stiffness	9	4
Albuminuria	20	8
Conjunctival injection	27	21
Duration before patient's admission to hospital	2·1 days	5·5 days
Total duration of fever	5·2 days	11 days
Duration of fever after chloramphenicol	62 hours (12 cases)	54 hours

Because of its relative frequency, and its severity if not treated with a suitable antibiotic, scrub typhus is the most important disease to be distinguished. Table III compares the clinical features of this series of leptospirosis and a series of 24 cases of scrub typhus collected during 1953.

The following facts are evident:

1. A group of febrile symptoms is common to both—for example, headache, abdominal pain and the symptoms of "feverishness", "shivers" and "sweating".

2. There are a number of features more frequent in leptospirosis—namely, limb pain, backache, vomiting, albuminuria. In these series the differences in the first three are statistically significant, the observed differences being greater than twice the standard error in all three, and greater than three times the standard error in two. However, they are of little value in the individual case, as all four are found in a considerable fraction of each series.

3. An eschar occurred in about one-third of the series of cases of scrub typhus, and when present established the diagnosis.

4. The presence of a rash was a valuable diagnostic feature, as it was very much more frequent in scrub typhus. It must, of course, be remembered that a rash can occur in leptospirosis.

5. The duration of illness from onset to admission to hospital is widely used as a differential feature; the difference was highly significant on the present figures. However, it must be recalled that scrub workers are more likely to be remote from hospitals. Fourteen patients of 22 with scrub typhus were ill six days or more before their admission to hospital, but only three of 113 with leptospirosis.

6. A history of exposure to scrub, swamp or canefields is of much diagnostic value. Certain hyperendemic areas of scrub typhus infection—for example, Yarrabah, Bramston and Mission beaches—are well known, and any patient with exposure there would be looked on as having a presumptive typhus infection. However, such considerations may cause confusion in individual cases; for example, several patients have caught leptospirosis after visiting the Bramston Beach area, the incidence of leptospirosis in scrub workers has been mentioned previously (Derrick *et alii*, 1954), and one caneworker at least caught scrub typhus while denying any scrub exposure, although it was later found that his farm adjoined scrub.

7. It is found that, while in many cases the provisional diagnosis on the patient's admission to hospital is correct, the response to treatment provides a further diagnostic criterion. Ten patients with scrub typhus were initially treated with penicillin on an incorrect provisional diagnosis; in eight cases obvious failure to respond led to the correct diagnosis and treatment. Two patients, admitted to hospital late in their course, appeared to respond. The converse error is less easy to detect; 12 patients with leptospirosis, treated with chloramphenicol became afebrile in an average time only slightly greater than the average in scrub typhus (sixty-two hours against fifty-four hours).

The proportion of cases of leptospirosis in which the correct diagnosis was made on the patient's admission to hospital was a little over 70% in this series; a correct diagnosis was reached in almost 90% before the patient's discharge from hospital. Only 13 of 24 cases of scrub typhus were recognized initially; all but two were correctly diagnosed before the patient's discharge from hospital. These figures emphasize that a clinical diagnosis, unsupported by laboratory proof, must be accepted with reservation.

Repeat Infections

A number of patients in the series claimed to have had previous similar illnesses, variously described as "coastal fever", "dengue" *et cetera*. While such a history is accepted with caution, definite evidence was found in some instances that the illness under investigation was not the patient's first bout of leptospirosis.

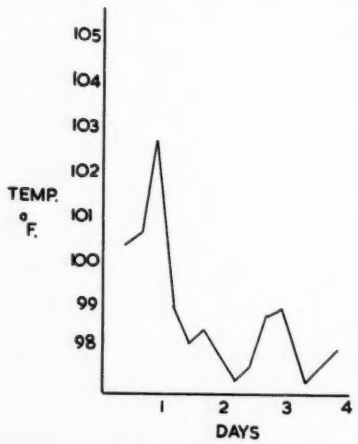

FIGURE III

Temperature chart in leptospirosis: no antibiotic therapy. Patient had a *hyos* infection; benign course may perhaps be correlated with *australis B* infection six months earlier

Thus, one patient is represented twice in this series (*australis B* and *australis A* infections), and another had *australis B* leptospirosis and scrub typhus within the twelve months preceding the *hyos* infection which is included here. Yet another had leptospirosis in 1950 diagnosed by agglutination as due to *australis B*; the "Szwajizak" infection which brought him into this series occurred in 1953, and at that time the agglutinins in his "acute" serum against "Robinson" rather indicated that the earlier infection was in fact caused by that type (not recognized in 1950). A fourth patient had an *australis B* infection proved in 1937. In addition, there were 13 patients who had agglutinins in their "acute" sera suggesting a previous attack of leptospirosis.

This is of considerable interest, as in the pre-antibiotic era convalescent serum was considered of therapeutic value. In most of the 17 cases, the clinical course does not appear to have been modified by the presence of heterologous antibodies; however, in one case, the *hyos* infection referred to above, the course was extremely mild. The patient was febrile for only two days and received no antibiotic therapy. The temperature chart is shown in Figure III. It would seem likely that he had a degree of immunity. Evidence of repeat infections within the same serogroup was not observed, either in the *pyrogenes* group (*australis B* and "Robinson") or in the *hebdomadis* group ("Kremastos" and "Szwajizak").

All 17 patients with evidence of previous infections were permanent residents of the area, and had spent at least several years there.

Treatment

Conflicting views have been expressed as to the value of the various antibiotics in leptospirosis. Abrahams *et alii* (1941) concluded from in-vitro tests that *icterohæmorrhagiæ* was relatively insensitive to penicillin. However, Heilmann and Herrell (1944) showed that penicillin would protect guinea-pigs against infection with *icterohæmorrhagiæ*. Alston and Broom (1944) had similar findings; both groups emphasized that penicillin was of no value if administration was delayed until the animals were sick.

Smith (1949) wrote as follows:

> There can be no doubt about the bactericidal and bacteriostatic action of penicillin on leptospira. It would seem that penicillin must be administered within the first four days, when the septicæmic stage is present; otherwise the action of the drug can avail the patient little when a toxic nephritis has developed.

Broom (1951) reviewed 206 cases of Weil's disease treated with penicillin and compared them with an earlier series; he concluded that penicillin did not significantly affect the mortality. However, it is mentioned that details of dosage and of the day of disease on which treatment was begun were available for a minority only.

Rosenberg (1951) wrote that "penicillin has definite value in the treatment of *canicola* infections".

Hall *et alii* (1951) reported an outbreak of 67 cases in Puerto Rico, which they divided into groups treated with different antibiotic regimes. They could not demonstrate any effect on the clinical course. Broom (1953*a*) has criticized these findings on the basis of the small numbers receiving each antibiotic.

A recent assessment of the position may be quoted (Broom 1953*c*):

> I have already mentioned that penicillin apparently does not reduce the death rate in classical Weil's disease but I have noticed that physicians very often record clinical improvement after its administration. I think we shall never be able to assess the true worth of antibiotics until we can analyse the results in a series of cases treated from the onset.

Comparison with Previous North Queensland Series

Two previous accounts of the clinical features of leptospirosis in North Queensland have been published; they form an interesting comparison with the present series, as both date from pre-antibiotic years. Morrissey (1934) reported 153 cases; 21 of the patients became jaundiced and seven died. Johnson (1950) described 35 cases of the "canefield leptospiroses"; nine of the patients were jaundiced and two died. In the present series of 115 cases there were no deaths; jaundice was suspected in four.

In the 1934 series, diagnosis in most cases was made on clinical grounds. *Australis A* and *australis B* were isolated at that time, and it seems reasonable to assume that they were responsible for the epidemic. However, other serotypes may have been present.

Johnson's series comprised six infections with *australis A* and 29 with *australis B* (all proven serologically). In comparing the present series, allowance must be made for the fact that it includes infections with five types of leptospiræ not recognized in Australia prior to 1951 (*canicola*, "Robinson", "Celledoni", "Kremastos" and "Szwajizak"). The 38 cases of *australis A* infection and the 29 of *australis B* infection form a more strictly comparable group. In Johnson's series the average durations of fever in the two groups were 8·2 and 10·9 days respectively; in the present series the durations were 4·5 and 5·8 days. It must be mentioned that some of the patients in Johnson's series received penicillin with results described as "encouraging". It was used in doses "approximating 500,000 units daily", which is a smaller dosage than those used in the present series.

The presence of the newer serotypes is a factor that may have influenced the overall clinical picture. Seven cases were due to *hyos* and two to *pomona*; these are known as benign strains from series of patients treated in the pre-antibiotic era, and it might be expected that in these nine cases the disease would have run a mild course even without treatment. No such basis for comparison exists with "Celledoni", "Kremastos" and "Szwajizak", and "Celledoni" cannot be compared to other known leptospiræ, as it appears to be a new type. "Kremastos" and "Szwajizak" are both members of the *hebdomadis* group which, when investigated previously (for example, in Japan), has been associated with a mild illness and very low mortality (Gsell, 1952, quoting Vaucel, mentions one death).

"Robinson" appears to resemble *australis B* very closely, serologically and in its pathogenicity for the guinea-pig. It must be linked with *australis B* as a serotype potentially icterogenic in man. *Canicola* is a world-wide strain and has been studied overseas. Three deaths have been ascribed to it, and of a series of 198 patients (Rosenberg, 1951), 13% became jaundiced. If the *hebdomadis* group, "Celledoni", *hyos* and *pomona* are excluded, 83 cases are left caused by serotypes potentially icterogenic.

The present series, then, must be considered to show a milder clinical picture than previous Australian series, and to suggest that antibiotic therapy has some value in leptospirosis.

Treatment Groups

Detailed information on treatment was available for 111 cases. The series can be divided into the following six groups, based on the treatment given:—A: no antibiotics: eight cases; B: penicillin, 100,000 units three-hourly: 25 cases; C: penicillin in dosage intermediate between (B) and (D): 20 cases; D: penicillin, 500,000 units, three-hourly: 26 cases; E: chloramphenicol: 12 cases; F: chloramphenicol *plus* penicillin: 20 cases.

The distribution of the serotypes in the six groups is shown in Table IV.

In assessing the merits of rival therapeutic regimes, mortality rate and frequency of jaundice were of no value here. The criteria used were the total duration of fever in days, and the duration of fever in hours after the commencement of antibiotic therapy. In each case a secondary rise of temperature, if present, was not included. The figures thus obtained are shown in Table V.

Group A, comprising the patients who received no antibiotic therapy, is small; however, the average duration of fever is longer

TABLE IV

Cases of Each Serotype Allotted to Treatment Groups

Group	Treatment	Serotype					
		australis A	*australis B*	" Kremastos "	*hyos*	Others	Total
A	No antibiotics	1	4	—	1	2	8
B	Penicillin, low dosage ..	6	6	7	—	6	25
C	Penicillin, intermediate dosage	8	2	3	—	7	20
D	Penicillin, high dosage ..	13	6	—	5	2	26
E	Chloramphenicol ..	3	3	1	—	5	12
F	Chloramphenicol *plus* penicillin	5	6	2	1	6	20
Total	.. ..	36	27	13	7	28	111

than in any other group. It might be expected that only cases running a favourable course would be permitted to remain in this group, so they may be looked on as a selected series of mild cases. One, a *hyos* infection with a duration of two days, has been discussed above ; this patient had had an *australis B* infection six months previously, and may have had some degree of residual immunity.

Group B comprises 25 patients who received 800,000 units of penicillin daily. The serotype distribution is shown in Table IV and the average duration in Table V. Scatter of duration in this group is pronounced, as shown in Figure IV. The shortest febrile period was sixteen hours in a " Kremastos " infection, the patient having been admitted to hospital on the second day of illness. On the other hand, one patient was febrile for 164 hours after the commencement of therapy (the infecting serotype was *australis B*) ; three remained febrile for 132 hours (one infection was due to *pomona* and two were due to " Robinson ").

Group C includes 20 patients who received penicillin in dosages between the 800,000 units per day of Group B and the 4,000,000 units per day of Group D. The durations, as shown in Figure IV, show much less scatter than in Group B.

Group D has 26 patients treated with penicillin, 4,000,000 units per day. Scatter of durations as shown in Figure IV is again much less than in Group B, although there is one extreme value (124 hours after therapy in an *australis A* infection). The shortest duration was eight hours after treatment ; the patient was a woman with a *hyos* infection, who was admitted to hospital on her fourth day of illness.

Group E includes 12 patients treated with chloramphenicol. The total dosage ranged from six to 14 grammes and the daily dosage from 1·5 to 3·0 grammes ; in several cases an initial loading dose of 1·0 to 3·0 grammes was given. Scatter of durations in this group is very pronounced, as shown in Figure IV. The shortest duration of fever after treatment was twenty-four hours in an *australis A* infection, the patient having been admitted to hospital on the eighth day of illness ; the longest

TABLE V

Duration of Illness in the Six Treatment Groups

Group	Antibiotic Treatment	Number of Cases	Average Duration Before Antibiotic Therapy Commenced (Days)	Total Duration of Fever (Days)			Duration of Fever after Antibiotic Therapy Commenced (Hours)		
				Average	Range	Standard Deviation	Average	Range	Standard Deviation
A	Nil	8	—	6·2	2 to 8	1·7	—	—	—
B	Penicillin (low dosage)	25	2·0	5·4	2 to 10	2·1	70	28 to 164	38
C	Penicillin (intermediate dosage)	20	2·6	4·75	3 to 9	1·1	39·2	12 to 120	23·5
D	Penicillin (high dosage)	26	2·5	4·3	3 to 9	1·7	34	8 to 124	27
E	Chloramphenicol ..	12	3·0	6·0	4 to 8	0·77	62	24 to 120	28
F	Chloramphenicol *plus* penicillin ..	20	2·0	5·4	3 to 7	1·1	72	24 to 124	26

duration after treatment was 120 hours in a *canicola* infection.

Group F is a heterogeneous one, comprising 19 patients who received both chloramphenicol and penicillin, and one who received "Aureomycin" and chloramphenicol. Many of these were considered to have scrub typhus, either on admission to hospital or when response to penicillin was not quickly apparent. Thus, in some cases the antibiotics were administered

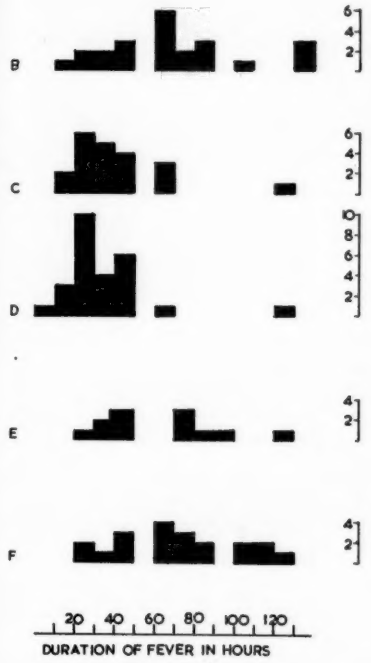

FIGURE IV

Duration of fever after antibiotic treatment. Ordinate: number of cases; abscissa: duration in hours. Group B received penicillin in low dosage, Group C penicillin in intermediate dosage, Group D penicillin in high dosage, Group E chloramphenicol, Group F penicillin and chloramphenicol

at the same time, in some penicillin was administered before chloramphenicol, in some chloramphenicol was administered before penicillin. As the indication for a change in antibiotic was in many cases an apparent failure of the initial therapy, this group would be expected to contain a selection of the more severe cases, or at least of those in which fever was prolonged. This may account for the long duration of fever in this group (seventy-two hours after therapy, the longest average duration).

Results of Treatment

As the patients were treated at eight hospitals, it cannot be claimed that their treatment was identical in every other respect than with regard to antibiotics. Hospital regimes varied within narrow limits in such matters as the frequency with which temperatures were recorded and the use of antipyretics. Each hospital tended to use one standard antibiotic regime, and thus all but one patient of Group D came from Babinda, and all of Group B from Innisfail. It was impossible to analyse any bias due to the hospital, as separate from that due to the antibiotics. The possibility that some other variable may be operative seems unlikely, but can be excluded only by future work.

The groups do not differ significantly in the stage of the disease at which treatment was commenced. In all treatment was commenced early, and although several groups contain individual cases in which treatment was delayed, this would have little effect on the figures. Also, consideration of both total duration of illness and duration after treatment would tend to neutralize any such bias.

All groups contain some patients who recovered quickly—that is, who appeared to respond to whatever treatment they received. It is probable that a number of these would have recovered as quickly without any antibiotic. However, it is surely suggestive that, of the 28 patients who became afebrile in under thirty hours after treatment, no less than 22 belonged to Groups C and D—that is, those receiving the higher dosages of penicillin.

Similarly all the groups contain at least one patient who, by a long duration of fever, appeared not to respond to antibiotic therapy. It must be pointed out that in some of these the secondary rise of temperature may have been included with the primary fever and so biased the figures. However, of the 25 patients who remained febrile for more than seventy hours after treatment, only two belonged to Groups C and D.

The total duration of illness is lowest in Group D, the group receiving the largest dosage of penicillin. This duration is significantly shorter than that of Group B, the observed difference of 1·1 days being twice the standard error of the difference.

Similarly the duration of illness after therapy is shortest in Group D, and this group has a duration significantly shorter than that of Group B (the observed difference of thirty-six hours being almost four times the standard error of the difference).

Group C has durations intermediate between Groups B and D, just as its penicillin dosage lies intermediate between them. The duration of fever after therapy in Group C is significantly less than that in Group B, when similar criteria to those above are used.

It is demonstrated, then, that as the penicillin dosage increases the duration of fever decreases, and that this decrease is greater than would be expected from statistical chance. However, before the shortening of duration can be ascribed to the antibiotic, the possibility must be excluded that the differences observed may be due to the varying serotype representation in the groups. As shown in Table IV, Group B contains a larger proportion of " Kremastos ", and Group D of *australis A* and *hyos*. The average febrile period for *australis A* infections for the whole series was 4·5 days, for *hyos* 3·7 days, for " Kremastos " 5·0 days, and for *australis B* 5·8 days. The problem is to decide whether the shorter duration of fever in Groups C and D was due to the larger number of *australis A* and *hyos* infections included, or whether the lower duration of fever in *australis A* and *hyos* infections was due to the higher proportion of these patients that received the larger doses of penicillin.

STATISTICAL ANALYSIS

Conclusions drawn from the statistical analysis undertaken by Mr. P. B. McGovern, M.A., B.Sc.,[1] are as follows:

Conclusions Drawn

If cases of *australis A* and *australis B* infection are each considered separately, the duration of fever after the commencement of therapy is significantly longer in each case in the group receiving the low dosage of penicillin than in the intermediate and high dosage groups. This difference is significant at the 5% level in each case. Thus, the difference mentioned above between Groups B and D is still present, and significant, if consideration is limited only to cases of *australis A* infection or of *australis B* infection.

The duration of fever after treatment is significantly longer in the group receiving both antibiotics than in the intermediate and high dosage penicillin groups. In *australis A* infections this is significant at the 1% level; in *australis B* at the 5% level. This is more difficult to evaluate, as one would expect the group receiving both antibiotics to contain cases with a longer febrile period (as discussed above). The value of chloramphenicol in leptospirosis cannot be assessed with confidence from the present series, as the number of patients who received it alone is small, and the patients who received both antibiotics are heterogeneous.

The durations of fever after therapy for *australis A* and *australis B* do not differ significantly.

The total duration of fever in *australis B* infections is significantly greater than that in *australis A* infections, independently of the effects of therapy. This difference is seen in the present series and in the series described by Johnson (1950).

The total duration of fever for the individual serotypes did not show significant differences between low and high penicillin dosages. However, significant differences were demonstrated for *australis A* and *australis B* infections between patients who received no antibiotic and the groups receiving intermediate and high doses of penicillin. Here, too, the mixed antibiotic group had a duration of fever significantly higher than the higher penicillin dosage groups. As above, this must be interpreted cautiously, as other factors may have loaded the mixed group with a selection of the more severe cases.

To summarize: the shorter duration of fever in the patients given the larger dosages of penicillin appears to be statistically significant, and to hold for individual serotypes, so far as that could be tested. It seems reasonable to claim that penicillin, in those dosages, and given early (as in the present series), does influence the clinical course of leptospirosis. The benign course of the series as a whole suggests that the smaller dosages of penicillin, even if suboptimal, may have some value. Opinion of the practitioners in the area is strongly in favour of penicillin therapy. Leptospirosis has been recognized in North Queensland for twenty years, and it is generally agreed that deaths from leptospirosis, as were seen in the 1930's and early 1940's, are now very rare. It must be emphasized that the practitioners here are well aware of the disease and that the standard of clinical diagnosis is high. The early stage at which treatment is begun must contribute largely to the favourable results.

[1] Mr. P. B. McGovern, of the Queensland Department of Agriculture and Stock, made the detailed statistical analysis, on which many of the conclusions are based. Mr. McGovern's mathematical treatment of the data was originally included as an " Addendum " to the paper, but it has been omitted at the request of the Editorial Committee. Copies may be obtained on application to this Institute.

Summary

One hundred and fifteen cases of leptospirosis are reported and their clinical features discussed. The mortality was nil, and no patient had definite jaundice, although its presence was suspected in four. The most frequent symptoms were headache, pain in the limbs, back or abdomen, vomiting, and photophobia. Abnormalities found most often on physical examination were enlargement of lymph glands, liver tenderness and conjunctival injection.

The average duration of fever was 5·2 days; this figure varied with the different serotypes, the duration being significantly longer in *australis B* infections than in *australis A* infections. Over one-third of the patients had a secondary rise in temperature which lasted from one to four days.

The differential diagnosis of leptospirosis in North Queensland may present difficulties, especially from scrub typhus. The features of greatest value in distinguishing the two diseases were the presence of an eschar or rash, the duration of illness before the patient's admission to hospital, a history of exposure to infection, and the response to treatment.

Seventeen of the series had previous leptospiral infections. In one case the mild clinical course of the second illness suggested that some residual immunity might have been present.

Comparison of this series with pre-antibiotic Australian series suggests that the clinical picture is milder in the present cases.

Analysis of the treatment given to 111 patients of the series indicates that penicillin has a definite therapeutic action, which is most pronounced in high dosages.

Acknowledgements

The writer is indebted to the practitioners of North Queensland for their assistance and cooperation, and especially to Dr. R. Hay, formerly of Babinda, who advocated the high dosages of penicillin and whose patients make up most of that group; to Mrs. M. Macgregor, librarian of the Queensland Institute of Medical Research, for much help with references; and to Mr. P. B. McGovern, M.A., B.Sc., for the statistical analysis. He also thanks Dr. E. H. Derrick for his generous help with all aspects of the work and with the preparation of this paper.

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LEPTOSPIRAL INFECTION IN NATIVES OF THE TERRITORY OF PAPUA AND NEW GUINEA[1]

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The purpose of this paper is to state the findings of an investigation undertaken with the object of determining the occurrence of leptospirosis in the native population of the Territory of Papua and New Guinea. Johnson (1953) states that " in the South-West Pacific area, leptospirosis has been diagnosed in Indonesia, Australia, Samoa, Hawaii and in certain islands in Micronesia, such as Truk and Ponape ". As far as we are aware, occurrence of the disease in the Territory of Papua and New Guinea has not been recorded previously. On the other hand, endemicity of leptospiral infection in the neighbouring land areas of North Queensland and Indonesia has long been confirmed. It is of immediate interest to refer, briefly, to the serotypes of leptospires which have been identified in these countries.

It is known that at least 11 distinct serotypes are responsible for human infections in North Queensland. This series includes the following members: *Leptospira icterohæmorrhagiæ*, *L. canicola*, *L. australis A*, *L. australis B*, " Robinson " strain, *L. hyos* syn. *mitis* (Johnson), *L. pomona*, *L. medanensis* (Ives strain) and the " Szwajizak ", " Kremastos " and " Celledoni " strains. The " Robinson " strain appears to be a new serotype of the *pyrogenes* serogroup, which includes *L. australis B*; the " Szwajizak " and " Kremastos " strains may represent entirely new serotypes of the *hebdomadis* serogroup, of which *L. medanensis* (Ives strain) is also a member; classification of the " Celledoni " strain is dependent on further antigenic analysis, but it may prove to be a new world entity (Smith *et alii*, 1954).

Wolff and Broom (1954) record the identification of 19 serotypes in Indonesia. Included in this series of serologically distinct strains are, *inter alia*, the following: *L. icterohæmorrhagiæ*, *L. australis A*, *L. pyrogenes*, *L. pomona*, *L. medanensis*, *L. hebdomadis*, *L. grippotyphosa*, *L. bataviæ*, *L. autiumnalis* (Rachmat strain), *L. andamana* (Collier, 1948) and *L. canicola* (Wolff *et alii*, 1951).

[1] Received on October 18, 1954.

The occurrence of these serotypes in North Queensland and Indonesia was taken into consideration in the selection of antigens for serological tests.

Sources of Material

Samples of blood were collected during 1953–1954 by venipuncture from 327 healthy male natives recruited for service in the Pacific Islands Regiments. The natives represented 11 Administrative Districts of the Territory of Papua and New Guinea, as follows: New Britain, 74 persons; Sepik, 54; Morobe, 49; Northern District, 33; New Ireland, 28; Milne Bay, 25; Central District, 21; Madang, 17; Gulf, 15; Western District, nine; Bougainville, two.

Serum was separated from whole blood in the Territory, forwarded by air transport to the School of Public Health and Tropical Medicine, Sydney, and stored in a deep-freeze unit pending serological examination. No preservatives were used.

Methods

The agglutination-lysis technique, described by Schüffner and Mochtar (1927), was used in this survey. Antigenic suspensions consisted of well-grown living cultures of leptospires in Schüffner's medium, each containing approximately 100,000,000 bacteria per millilitre. Serial dilutions of the serum under investigation were mixed with equal volumes of antigen, so that final dilutions of the serum followed the series 1/30, 1/100, 1/300, 1/1000 *et cetera*. The mixtures of serum and antigen were incubated at 30° C. for three hours and examined microscopically by dark-ground illumination, with the use of a 10× objective and a 10× or 15× eyepiece. This procedure is substantially the technique recommended by Wolff (1953).

Each serum was tested against a series of 19 antigens, representing the following leptospiral serotypes (strain designation in parentheses): *L. icterohæmorrhagiæ* (Jackson), *L. pomona* (Staines), *L. hyos* syn. *mitis* (Johnson), *L. grippotyphosa* (Moscow V), *L. australis A* (Ballico), *L. australis B* (Zanoni), *L. canicola*

(Berlin), *L. hebdomadis* (Hebdomadis), *L. medanensis* (HC), *L. medanensis* (Ives), *L. pyrogenes* (Salinem), *L. bataviæ* (Se 62), *L. autumnalis* (Autumnalis), *L. bangkinang* (SC 60), *L. andamana* (CH–11) and the "Robinson", "Szwajizak", "Kremastos" and "Celledoni" strains.

Cultures of *L. pomona* (Staines), *L. medanensis* (Ives), "Robinson", "Szwajizak", "Kremastos" and "Celledoni" strains were made available by Dr. J. I. Tonge, Laboratory of Microbiology and Pathology, Brisbane. The remaining cultures were provided by Dr. J. C. Broom, of the Wellcome Laboratories of Tropical Medicine, London.

Cultures were tested regularly for antigenic stability against homologous immune rabbit sera prepared at the Wellcome Laboratories of Tropical Medicine, London, and at this School.

In parallel with the conventional agglutination-lysis technique, some sera were examined by a modification of the rapid slide method described by Krüger (1953). Findings of the two methods showed close conformity and will be included in a separate publication by one of us (J.S.W.).

RESULTS

It is generally agreed that a positive agglutination response, even at low titre, provides specific evidence of current leptospiral infection or of residual antibodies due to past disease. Moreover, antibodies may be demonstrable in serum for many years after an attack of leptospirosis (Broom, 1948). The agglutination test is, consequently, a valuable laboratory procedure for detection of leptospiral infection in a human or animal population.

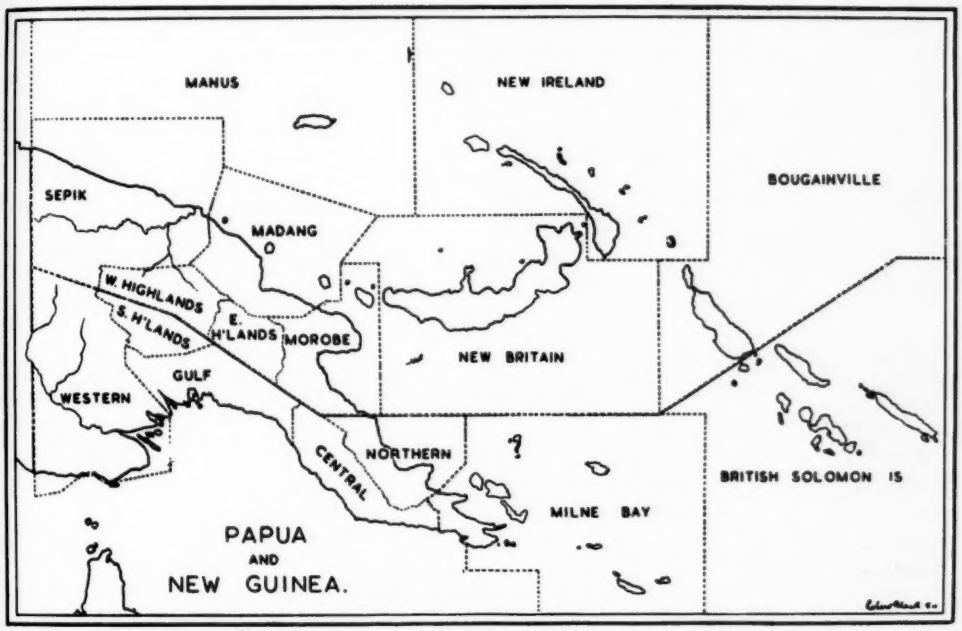

FIGURE I

Map showing the Administrative Districts of the Territory of Papua and New Guinea

In a survey of workers exposed to occupational hazard, Ward and Turner (1942) regard an agglutination titre of 1/10 as significant of past infection with leptospires. Stuart (1946) states that he has never detected an agglutination titre of 1/10 in persons who have not been exposed, at least, to a probable risk of infection. Broom (1948) records detection of leptospiral antibodies, at titres of 1/10 or 1/30, in 23 of 875 persons suffering from unrelated diseases. However, most of these positive reactors were employed in occupations which implied the possibility of previous leptospiral infection.

We have been concerned with the problem of determining the occurrence of leptospirosis in

an area in which the disease has not been previously recorded. For this purpose we have elected to regard agglutination-lysis in a serum dilution of 1/100 or higher as a positive reaction.

TABLE I

Reactions of 327 Sera Against 19 Leptospiral Antigens

Sera	Number	Percentage of 188 "Positive" Sera	Percentage of All Sera
Sera reacting exclusively against a particular serogroup or serotype at a titre of 1 : 100 or higher. No co-agglutinins detected at any titre[1] ..	65	34·6	19·9
Sera reacting predominantly against a particular serogroup or serotype at a titre of 1 : 100 or higher, and showing in addition agglutinins against other serotypes[2]	123	65·4	37·6
Sera failing to react against a serogroup or serotype at any titre	139	—	42·5
Total	327	100	100

[1] See Table II for detailed analysis.
[2] See Table III for detailed analysis.

Of 327 sera tested, 188 (57·5%) have been found to react against one or more of the 19 leptospiral antigens employed, in serum dilutions varying from 1/100 to 1/1000.

Analysis of the 188 positive findings has shown that they can be included, broadly, in the following two categories: (i) sera which contain antibodies exclusively to a particular serogroup or serotype, at a titre of 1/100 or higher, but which do not react against any other antigen; (ii) sera which contain antibodies, predominantly to a particular serogroup or serotype, at a titre of 1/100 or higher, but which react, also, at lower titre, against an unrelated antigen or antigens. A small percentage of these sera reacted, in identical dilutions, against one or more members of a serogroup and an unrelated antigen.

The first category includes 65 sera, representing 34·6% of "positive" sera and 19·9% of all sera tested; the second category includes 123 sera, representing 65·4% of "positive" sera and 37·6% of all sera tested. These findings are shown in Table I.

In Table II is set out an analysis of the 65 sera in the first category. The general pattern of a list of recognized serotypes, suggested by Wolff and Broom (1954), has been followed in the classification of reactions. The "Szwajizak" and "Kremastos" strains have been included in the *hebdomadis* serogroup, the "Robinson;' strain in the *pyrogenes* serogroup. The "Celledoni" strain has been listed as an individual serotype. Administrative Districts of the

TABLE II

Series of 65 Sera Reacting Exclusively Against a Particular Serogroup or Serotype at a Titre of 1 : 100 or Higher; No Co-agglutinins Detected at any Titre

Serotype	Serotype[1]	Districts[2]	Number of Sera	Percentage of 188 "Positive" Sera
Hebdomadis	*hebdomadis* (Hebdomadis), *medanensis* (HC), *medanensis* (Ives), "Szwajizak", "Kremastos"	All districts with the exception of Bougainville	58	30·9
Pyrogenes	*pyrogenes* (Salinem), *australis B* (Zanoni), "Robinson"	New Britain	1	0·5
Autumnalis ..	*autumnalis* (Autumnalis), *bangkinang* (SC 60)	Milne Bay, Morobe	2	1·1
Australis A	*australis A* (Ballico)	——	—	—
Bataviæ	*bataviæ* (Se. 62)	Gulf	1	0·5
Icterohæmorrhagiæ	*icterohæmorrhagiæ* (Jackson)	——	—	—
	hyos syn. *milis* (Johnson)	Northern, Western	2	1·1
	andamana (CH–11)	Western	1	0·5
	grippotyphosa (Moscow V)	——	—	—
	canicola (Berlin)	——	—	—
	pomona (Staines)	——	—	—
	"Celledoni"	——	—	—
Total ..	.. ..	.. ..	65	34·6

[1] Strain name in parentheses.
[2] As listed in text.

TABLE III

Series of 123 *Sera Reacting Predominantly Against a Particular Serogroup or Serotype at a Titre of* 1 : 100 *or Higher, and Showing, in Addition, Agglutinins Against Other Serotypes*

Serogroup	Serotype[1]	Districts[2]	Number of Sera	Percentage of 188 "Positive" Sera
Hebdomadis	*hebdomadis* (Hebdomadis), *medanensis* (HC), *medanensis* (Ives), "Szwajizak", "Kremastos"	All districts with the exception of Bougainville	78	41·5
Pyrogenes	*pyrogenes* (Salinem), *australis B* (Zanoni), "Robinson"	New Ireland, Sepik, Milne Bay, Madang, New Britain, Northern	15	8·0
Autumnalis ..	*autumnalis* (Autumnalis), *bangkinang* (SC 60)	New Britain, Madang	2	1·1
Australis A[1] ..	*australis A* (Ballico)	Sepik, Gulf, Western, Madang	7	3·7
Bataviæ	*bataviæ* (Se. 62)	Morobe	1	0·5
Icterohæmorrhagiæ	*icterohæmorrhagiæ* (Jackson)	New Britain, Central, Milne Nay, Madang	4	2·1
	hyos syn. *mitis* (Johnson)	New Ireland, Milne Bay, New Britain, Northern, Sepik	6	3·2
	andamana (CH–11)	——	—	—
	grippotyphosa (Moscow V)	Sepik, Western	3	1·6
	canicola (Berlin)	Milne Bay	1	0·5
	pomona (Staines)	Central, Sepik	2	1·1
	"Celledoni"	Central, Sepik	4	2·1
Total ..	.. ..	.. ..	123	65·4

[1] Strain name in parentheses.

[2] As listed in text.

Territory of Papua and New Guinea, from which sera were derived, are also shown.

Table III represents a similar analysis of the 123 sera in the second category, including serogroups and serotypes against which reactions were recorded and geographical areas to which sera could be related.

DISCUSSION

As was stated above, critical evaluation of the agglutination test for leptospirosis has demonstrated its specificity. It can be reasonably assumed that the result, even at low titres, indicates past infection with leptospires.

Of 188 positive reactions recorded in this study, 14 (7·5%) indicated antibodies to one or other antigen at a titre of 1/1000, 61 (32·5%) at a titre of 1/300 and 113 (60%) at a titre of 1/100. It is considered that these findings provide adequate evidence of the occurrence of leptospirosis in the Territory of Papua and New Guinea. Positive reactors were detected in all Administrative Districts included in the survey with the exception of Bougainville, which was represented by two sera only.

Whilst a positive serological finding indicates leptospiral infection, past or present, it does not necessarily identify the serotype responsible for the infection. Isolation of the infecting strain is desirable for conclusive determination of its identity. However, examination of Table II shows that 58 (30·9%) of 188 "positive" sera reacted exclusively against members of the *hebdomadis* serogroup. Furthermore, Table III indicates that an additional 78 (41·5%) of the 188 positive sera reacted at highest titre against members of the same serogroup. Thus, in all, 136 (72·4%) of "positive" sera contained antibodies to one or more of the *hebdomadis* serotypes employed as antigens in the tests. No attempt was made to identify the causal serotype more completely by cross-absorption tests. However, the serological evidence does suggest that some member or members of the *hebdomadis* serogroup are associated with human infections in the Territory.

Evidence relating to the incidence of serotypes, other than those of the *hebdomadis* serogroup, is by no means so impressive. Nevertheless, it does at least suggest the occurrence of several serotypes in the Territory, and perhaps the possibility of successive infections with heterologous serotypes.

Concerning human infections with *L. hebdomadis* in the Andaman Islands, Barker (1926) makes the following statement:

> The disease is characterized by sudden invasion, severe headache, muscular pains in the back and limbs, and pyrexia lasting 6–7 days, usually with a "saddle-back" curve and associated with a pulse which is relatively slow in relation to temperature.

The eyes are congested, and there may even be conjunctivitis; lymphatic glands are enlarged; digestion is upset and the patient suffers from much depression. There is no rash in the Japanese cases, no jaundice and no relapse. The disease is really a mild one, compared with the other leptospiral diseases, and the mortality is nil.

In *hebdomadis*-type infection, according to Van Thiel (1948), "the picture of the disease is considerably milder than in Weil's disease. Icterus very seldom occurs. A cloudy corpus vitreum has occasionally been seen. As a rule no mortality."

We are informed (Doherty, 1954) that, to date, none of the *hebdomadis* type infections in North Queensland, due to the *medanensis*, "Szwajizak" or "Kremastos" serotypes, has been associated with jaundice, and none has been fatal. The average duration of illness in 13 cases of infection with the "Kremastos" strain has been 5·0 days; the average duration of illness in five cases due to the "Szwajizak" strain has been 5·8 days. It is considered that the clinical pattern of the disease may have been conditioned by early exhibition of antibiotic therapy.

If, as the serological findings seem to suggest, infections due to members of the *hebdomadis* serogroup are not uncommon, it is possible that leptospirosis in the Territory is often a relatively mild non-icteric disease. It is possible also that many infections do not reach the clinical level. On the other hand, there may also be cases, due to unrelated serotypes, or even to leptospires of the *hebdomadis* serogroup, in which jaundice develops, and which assume a more striking clinical character.

Having regard to the limited information available, it is neither practicable nor desirable to speculate further on the epidemiology of leptospirosis in the Territory. However, it may be stated, that, for the most part, rainfall is high in districts which, to judge by the serological findings, are areas of probable endemicity. There is, moreover, in these districts a population of field rodents and marsupials which may well serve as a reservoir of infection. It is of interest to note that, in North Queensland, *medanensis* agglutinins have been detected in a possum (*Trichosurus*), "Szwajizak" agglutinins in a bandicoot, and "Celledoni" agglutinins in a bandicoot. *L. medanensis* has been isolated from a dog in Sumatra (Derrick *et alii*, 1954). Although cattle are not numerous in the Territory, there are many domestic pigs in close association with human habitation. It may be noted that few sera examined in this survey contained either *pomona* or *hyos* antibodies.

It is proposed to continue this investigation with the immediate object of detecting reservoirs of infection in field rodents and marsupials. In addition, we propose to examine bovine, porcine and canine sera for leptospiral antibodies.

SUMMARY

A series of 327 sera from healthy natives in the Territory of Papua and New Guinea has been tested, by the agglutination-lysis technique, for reactions against antigens of 19 leptospiral serotypes.

Of these sera, 188 (57·5%) have been found to contain antibodies of one or more serotypes at titres varying from 1/100 to 1/1000. It is considered that this finding provides adequate evidence of the occurrence of leptospirosis in the Territory.

An analysis of the serological findings (Table II) has shown that 58 (30·9%) of the positive sera contained, exclusively, antibodies of the *hebdomadis* serogroup. In addition 78 (41·5%) of the positive sera reacted predominantly against antigens of the *hebdomadis* serogroup (Table III).

Serological evidence relating to the incidence of other serotypes is much less striking. It is possible, however, that a number of serotypes may be causal agents in human infection.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. J. T. Gunther, Director of Public Health, Territory of Papua and New Guinea, for approving the proposal to undertake this investigation and for enthusiastic cooperation in implementing the survey, particularly with regard to the collection of material.

Our thanks are due also to Professor Edward Ford and Dr. R. H. Black, School of Public Health and Tropical Medicine, Sydney University, for their interest in the work, and to the Director-General of Health, Canberra, for permission to publish this paper.

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PNEUMONIA[1]

R. B. LEFROY

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IT is often considered that the problem of pneumonia has to a large extent been answered since the advent of chemotherapy in the last two decades, and that if there is anything further to be done in this field it is simply a matter of countering the offensive of a particular organism with the latest discovery in antibiotic production. Yet in spite of a striking reduction in mortality, the fact that so many of our hospital beds are occupied by patients suffering from chest infections makes it evident that morbidity is still considerable, particularly in our aging population.

One of the many aspects worthy of consideration is the problem of classifying this somewhat nebulous heap of diseases which collectively are regarded as " the pneumonias ". Providing a label for a disease becomes an unnecessary by-product of medical investigation—in fact, it becomes a disease in itself—unless we are careful to devise a classification with respect to ætiology and pathogenesis. The current use of that popular term " atypical pneumonia " can scarcely be regarded as a useful description of any chest infection, particularly since our conception of typical pneumonia is a variable one. Scadding (1948) makes the following statement:

> The term " atypical " is logically indefensible. It presupposes that the observer has in mind some ideal norm of pneumonia which he regards as typical, and implies that the sort of pneumonia to which reference is being made is not like the norm. No one, however, has stated what this typical pneumonia is . . . In fact, this term, implying knowledge of an ideal and perfect pneumonia existing as it were in some platonic heaven, is not only meaningless but confusing.

The present chaotic position would seem to provide a reason for reviewing this problem.

It is difficult to appreciate the various factors involved in chest infections, or plan effective therapy, by the use of a classification built upon anatomical divisions, such as lobar pneumonia and bronchopneumonia. To regard this group of infections from a purely bacteriological viewpoint has the virtue of introducing specificity which is important; yet there is good reason not to remain satisfied with a classification based on a list containing practically any organism which has demonstrated its ability to descend into the lower part of the respiratory tract.

One hundred and ninety consecutive cases were recently studied with these problems in mind. This series excluded those patients whose infection followed pulmonary neoplasm, or was caused by the tubercle bacillus; nor were cases with the more specific label of bronchiectasis included. Yet another omission has been that important and largely preventable group of chest infections seen in the surgical wards in which the disease has followed operation. These patients have been omitted from the present study merely because the causal factors responsible for their episodes of chest infection have been, broadly speaking, beyond suspicion.

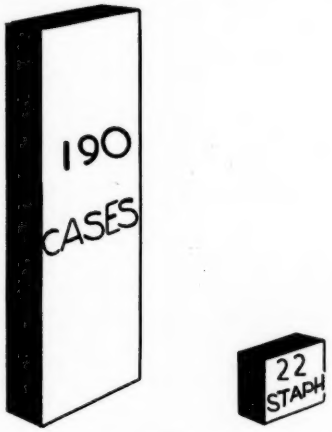

FIGURE I

Showing the proportion of patients from whose sputum *Staphy. aureus* was cultivated in a series of 190 admitted to the Royal Perth Hospital

Of the 190 cases of chest infection not belonging to any of the foregoing categories, in 22 a hæmolytic *Staphylococcus aureus* was

[1] Received on July 2, 1954.

TABLE I

Details of Four Cases of Staphylococcal Influenzal Pneumonia

Age (Years)	Date of Admission to Hospital	Symptoms at Onset	Symptoms on Admission to Hospital	Time in Hospital	Staphylococcus from
19	18.8.53	Sore throat, sore eyes, 24 hours	Retrosternal pain	22 hours	Throat swab, sputum, lung *post mortem*
16	26.8.53	Cough, malaise, 4 days; irrational, 16 hours	Dyspnœa, delirium, circulatory failure	10 hours	Spleen
17[1]	26.8.53	Sore throat, cough, 5 days	Chest pain, dyspnœa, cyanosis, failure of circulation	3 weeks	Sputum
16	31.8.53	Sore throat, headache, dyspnœa, 48 hours	Delirium, cyanosis, purpura	14 hours	Naso-pharynx, blood, lung *post mortem*

[1] The sole survivor.

grown on culture from the sputum. Our problem is to decide whether they should all be labelled as "staphylococcal pneumonia", or whether there are other features in each case which should call for a classification embracing a wider vision of ætiology than the mere name of an organism, however virulent, can suggest.

Within this group of 22 are four cases, which occurred during an influenza epidemic, and which represent a specific disease, vastly different from the others (Table I). All these patients were below the age of twenty years, and they were all admitted to hospital within a period of three weeks; in all except one case, in which the patient survived a stormy illness, the disease was rapidly fatal. A full description of these cases is published elsewhere (Lefroy and Keall, 1954). This relentless march of events occurring in a young adult, beginning with mild upper respiratory symptoms and soon progressing to an overwhelming degree of pneumonia and generalized toxæmia, is even in the absence of bacteriological studies very suggestive of a specific type of respiratory infection—influenzal staphylococcal pneumonia. Cultures of sputum and of lung secretion obtained *post mortem* provided proof of the presence of the staphylococcus. Proof that the influenza virus was also implicated is more difficult; the early symptoms and signs of upper respiratory infection cannot be regarded as pathognomonoc; nor was the virus recovered from the respiratory tract after death. However, consideration must be given to the finding of rising titres in the influenzal antibody level of serum taken from other patients in Perth at that time. Consequently, it is submitted that sufficient evidence has been obtained from clinical, bacteriological and pathological consideration, to regard this as a specific disease which, for want of a more precise label, can be referred to as influenzal staphylococcal pneumonia—a disease in which the combined action of influenza virus and staphylococcus produces under certain conditions an illness in young adults which is often fatal.

However, the object of this study is not to discuss the intriguing and important problems which have evolved from this freak among respiratory infections; it is used rather as an example of an infection whose characteristics have earned it a distinct and unique position among the varied host of respiratory diseases in general, and of those branded by the name of the staphylococcus in particular. It defies classification on a simple bacteriological basis—to call it staphylococcal pneumonia is to deny the important role played by the virus. To invoke the old system of anatomical division into lobar pneumonia and bronchopneumonia is likewise meaningless, and gives no indication of the subtle interconnexion between host, virus and bacterium which is responsible for the strange pathogenesis of the disease.

In contrast with this, yet still in the predominantly staphylococcal group, is one case of pneumonia complicating staphylococcal septicæmia.

CASE I.—A man, aged fifty-one years, had had an infected abrasion in the region of the left knee. About ten days later he began to complain of lassitude and fever, soon followed by pleuritic pain and cough with blood-stained purulent sputum. When he was admitted to hospital three weeks after the onset he was extremely ill, having lost weight and become obviously anæmic. Clinical examination of his chest suggested scattered areas of consolidation on both sides. He continued to remain in this emaciated condition with intermittent fever and chest pain, and a cough which at times produced copious purulent sputum tinged with blood, and at others only a scanty amount, until recovery gradually took place after a period of over two months. A *Staph. aureus*, hæmolytic and coagulase-positive, was repeatedly cultivated from the sputum, the blood and the infected skin abrasion. The organism was resistant to penicillin according to sensitivity tests *in vitro*, but sensitive to chloramphenicol, streptomycin and "Terramycin".

There can be no doubt that these drugs, given in a variety of combinations, made this patient's recovery possible, though no immediate or dramatic response was observed. Serial chest X-ray films taken during the time of his illness showed the wandering rounded shadows with the appearance of cavitation and cyst formation, at one time involving one part of the lung most severely, at another having quite a different anatomical pattern. It was many weeks after his convalescence was complete that X-ray films of the chest showed a normal appearance. This sequence is consistent with the well-known description of the disease given by Ryle (1930) and others. However, fortunately, pleural involvement was never serious, and this patient escaped the dreaded sequelæ of a broncho-pleural fistula or an established lung abscess.

Not only does this disease process bear the hall-mark of septicæmia—with its localized manifestations in skin, bone and lung—but from the appearance and behaviour of the pulmonary lesions it also shows itself to be characteristic of a staphylococcal infection. It would, therefore, seem correct to label this case as one of staphylococcal pneumonia—not merely because this organism appears in the sputum, but because of the specific nature of the clinical features.

CASE II.—A man, aged sixty-three years, ran a similar but less severe course, but differed from the patient in Case I in that there was no evidence of generalized pyæmia. After an onset which had neither the explosive character of the influenzal pneumonia of young adults, nor the association of an extrapulmonary staphylococcal lesion, this man was in hospital for over three months. A hæmolytic, coagulase-positive *Staph. aureus*, insensitive to penicillin by tests *in vitro*, was recovered in culture from the sputum on numerous occasions. Though he was never desperately ill, the patient lost weight and had intermittent rises of temperature during most of his stay in hospital. X-ray examination of his chest revealed irregularly shaped shadows in both lung fields, which varied from time to time both in their density and in their position. Over twenty years previously he had had an empyema, which was apparently successfully drained by rib resection at the base of the left lung. It does not seem beyond the bounds of possibility that the lung tissue in this area was permanently altered, particularly with regard to the anatomy of the smaller bronchi, and that in this region there began many years later the nidus of a staphylococcal infection which eventually involved the major portion of both lungs.

Whether or not this theory regarding the initial focus is acceptable, the course of the patient's recent illness, the serial radiographic appearances and the repeated findings on sputum culture provide a syndrome which can be truly called staphylococcal pneumonia.

The difference between that pulmonary disease in young adults occurring during the influenza epidemic, and the less dramatic but more prolonged illness experienced by the last two patients, need not be stressed further. We are left, then, to consider the differences and similarities which exist between the two types of infection already discussed, and those other respiratory infections among the 22 cases in which the staphylococcus was recovered.

There are several reasons why one should refrain from referring to this latter group as staphylococcal pneumonia. First is the fact that the mere recovery of a staphylococcus from the sputum does not necessarily mean

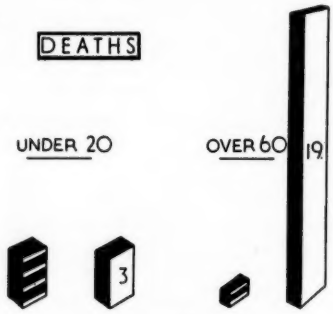

FIGURE II

An analysis of deaths occurring in two age groups of patients admitted to hospital with chest infections. The cross-hatched areas represent patients in whose sputum the staphylococcus was found. The plain columns represent the total number of deaths in each group

that it is causing an infection in the lower part of the respiratory tract. Secondly, the clinical course run by these older patients does not appear to depend on the particular organism which was recovered. This second point can be partly illustrated by a consideration of the deaths which have occurred and the number of times the staphylococcus has been involved in the 190 cases in the present series. In the younger age group all the deaths were associated with the staphylococcus, whereas in the over sixty years group, in which 19 patients died, only one produced sputum containing this organism (Figure II); since this patient died from a pulmonary embolus, one can hardly blame the staphylococcus entirely for his death. Nor was there evidence of the prolonged illness with a hectic fever and obvious constitutional signs, or of the radiographic findings of scattered lesions sometimes breaking down to cavity

formation and often lingering long into the phase of convalescence; these were not features of the remaining 16 cases in the so-called staphylococcal group. These patients had a less conspicuous illness whose course seldom caused concern (unless they carried the burden of age or some additional infirmity); their radiographs generally excited little comment, and they were eventually discharged from hospital bearing the label of "pneumonitis", "broncho-pneumonia" or some equally mystifying term, which scarcely hinted at the ætiology or pathogenesis of their illness.

While it is not admitted that the name staphylococcal pneumonia adequately describes such cases, it is not implied that the presence of a potentially pathogenic organism should be disregarded. However, to learn more of the exact pathogenesis of chest infections, note should be taken of factors other than the organisms cultivated from the sputum; therefore, a classification of pneumonia on a purely bacteriological basis is insufficient. Similarly, the treatment of the disease calls for other therapeutic weapons in addition to chemotherapy.

Stuart-Harris (1953) has shown that comparatively frequently the influenza virus is associated with cases of pneumonia which are clinically no different from those which have for so long been called pneumococcal or streptococcal pneumonia. Of this possible association in our own community we have no information, but it is obviously a line of research which must be followed wherever the ætiology of chest infection is seriously studied.

In addition to the part played by virus and bacteria, a factor of importance in both the understanding and the treatment of chest infections—in the elderly in particular—is the state of the bronchi and their ability to cause segmental obstruction. That this feature can be the major ætiological factor in some types of pneumonia is unquestionable when the following case is studied.

CASE III.—A man in his early sixties was admitted to hospital on several occasions because of recurrent infection associated with collapse in the upper lobe of the right lung. A bronchoscopic examination was performed on the last occasion and a fibroma was removed from the right upper lobe bronchus; this tumour, attached to the mucosa by a pedicle, was able to cause intermittent obstruction and thereby infection. X-ray examination of his chest on the last admission to hospital showed, in addition to the right-sided abnormality, a shadow in the left lung; it seems probable that this originated from a "spill over" from the focus on the right side, and consequently its presence was the indirect result of bronchial obstruction.

This case is quoted because it is an undoubted example of the association between bronchial abnormality, obstruction and infection, and shows that when one is considering the cause of infection, this factor takes precedence over the bacteriologist's findings in the sputum. The following case provides a less flagrant example of what is essentially the same pathological process.

CASE IV.—A man, aged fifty-two years, was admitted to hospital, having had an intermittent respiratory infection for about eight weeks; for most of this time he had had a productive cough, with purulent sputum. A short course of chemotherapy was given by his general practitioner four weeks after the onset, after which he returned to work. Lassitude, fever and cough were his principal complaints on his admission to hospital. Radiographic examination confirmed the clinical signs of extensive parenchymal involvement of the right lung; there was an overall loss of translucency throughout the lower lobe, the diaphragm being raised on that side; the lateral view confirmed the suspicion of partial collapse of the lower lobe; evidence of consolidation was also seen in part of the upper lobe. The patient was never in distress from this extensive pulmonary involvement. His temperature never rose above 100° F., and he felt reasonably well during his stay in hospital. Sputum culture on one occasion produced a *Staph. aureus*, hæmolytic and coagulase-positive. This organism was sensitive to penicillin *in vitro*, but although 600,000 units a day were given for a week, no difference was noticed in clinical signs or in the amount of sputum being produced.

One month after his admission to hospital, X-ray examination of the chest showed little change, and although bronchoscopic examination at this time revealed nothing more than some stenosis of the lower lobe bronchus, it was considered that the abnormality underlying this "unresolved pneumonia" was a bronchial carcinoma, with involvement of the phrenic nerve. The patient was discharged from hospital and followed in the out-patient department. An X-ray film of the chest taken three months after his discharge from hospital proved to be within normal limits.

There are numerous features of interest and importance in this case. First, apart from one positive sputum culture, there are none of the features, either clinical or radiographic, which we generally associated with staphylococcal pneumonia. Secondly, there is evidence that bronchial obstruction was present. It is possible that an attack of pneumonia eight years previously had left some derangement of the lower lobe bronchus, which with the insult of his recent respiratory infection became sufficiently narrowed to cause obstruction and in turn infection, not only of the entire lower lobe, but also—probably by aspiration—of part of the upper lobe. On the other hand, obstruction of the lower lobe may have arisen directly from a mucopurulent plug aspirated in the initial stages of his respiratory infection two months before his admission to hospital.

Thirdly, bronchoscopic examination was undertaken as a diagnostic measure; however, it was probably of considerable therapeutic value in aiding drainage from the lower lobe. Too often, as in this illness, bronchoscopy is undertaken when parenchymal disease is already well established.

It seems likely that this same train of events has occurred in other cases, even though positive clinical and radiological evidence of bronchial obstruction is lacking. Often the history gives evidence of an upper respiratory tract infection preceding the episode in the lungs by days or weeks, with the production of sputum quite capable of causing segmental

CASE V.—A frail woman, aged seventy-three years, had an upper respiratory infection which was followed four days later by a discharging ear; at the end of a fortnight she had, in addition, a productive cough and increasing malaise. When she was admitted to hospital three weeks after the onset, dyspnœa and cyanosis necessitated the use of oxygen. A pneumococcus was cultivated from the sputum, and she was given penicillin. Because there had been such little improvement on this regime, and because chest X-ray and clinical signs gave evidence of intermittent obstruction of the lower lobe of the left lung, bronchoscopy was carried out. Nearly 10 millilitres of mucopurulent secretion were aspirated with considerable relief to the patient, though a second bronchoscopy had to be performed before clinical and radiographic signs returned to normal (Figures III and IV). Postural drainage was begun as soon as her general condition permitted.

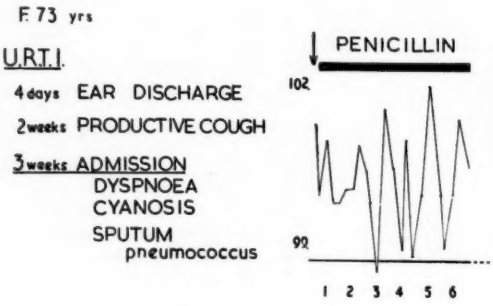

FIGURE III

Case V: the clinical course, showing daily temperature chart while penicillin was administered as the mainstay of treatment

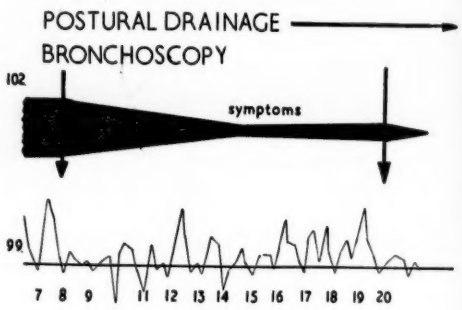

FIGURE IV

Case V: illustrating the value of bronchoscopy and postural drainage, when deficient bronchial drainage is a prominent feature

obstruction. More common, perhaps, in older people has been a story stretching back over many winters, punctuated with episodes of bronchitis which have caused little alarm; but each episode damages the bronchial mucosa a little more, so that in time it becomes inefficient in the performance of its physiological duties of defence, and at length becomes structurally deranged so that obstruction and retained secretion, leading to inflammation of the surrounding lung parenchyma, are the sequelæ of what would otherwise be a mild bronchial infection. The morbidity in these cases does seem to lessen when postural drainage is energetically undertaken, and when bronchoscopy is used as a therapeutic weapon as soon as it is apparent that the more conventional lines of treatment are ineffective.

The influence of bronchial obstruction on the pathogenesis of chest infection was observed to a lesser or greater degree in many of the 190 cases.

Although the pneumococcus was isolated, pneumococcal pneumonia cannot be regarded as a term which describes the essential features of this case. It is clear that from the point of view of therapy, improvement in her condition resulted more from bronchoscopy and postural drainage than from the administration of penicillin.

CONCLUSIONS

From this study of 22 patients in whose sputum the staphylococcus was found, it seems reasonable to draw certain conclusions regarding the classification of "pneumonia". It has been argued that in this small group the disease which was observed in young adults when the staphylococcus combined with the influenza virus had features which differed widely from the illness which the staphylococcus caused when the pulmonary disease was part of a septicæmia, and that whereas it is true to state that this organism was the

main ætiological factor in the latter instance, the remaining cases lack the specific characteristics of staphylococcal pneumonia, and thus form a third category within this group of 22 cases (Figure V). In this last category, one recognizes to a greater or lesser extent the effect of bronchial obstruction.

Nor are these findings peculiar to cases in which the staphylococcus was found in the sputum; they are surely relevant to that whole collection of chest infections which we refer to as the pneumonias (Figure VI). In some the presence of pathogenic bacteria is largely responsible for the characteristics and the pathogenesis of the disease. Mention has been previously made of the combined presence of influenza virus and bacteria in cases of pneumonia which were clinically no different from those which we regard as of purely bacterial origin (Stuart-Harris, 1953). In a third group are those infections due solely to the presence of virus: the psittacosis group, the viruses responsible for chicken-pox and smallpox and the closely related *Rickettsia burneti* are undoubted causes of pneumonia. But there

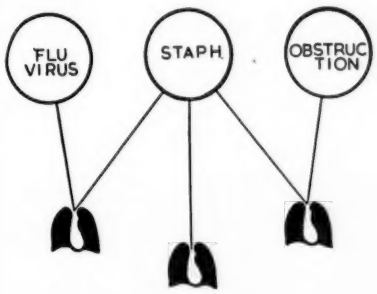

FIGURE V

Diagrammatic representation of the factors responsible for the chest infections outlined in the text

are many cases, often sheltering under the label of " primary atypical pneumonia ", whose inclusion in this group is not beyond suspicion.

It is the purpose of this paper to give prominence to the existence of yet another factor unconnected with virus or bacteria—the factor of bronchial obstruction. This feature in the ætiology of pneumonia has been eminently put forward by Scadding in his descriptions of aspiration pneumonia (1948 and 1952). It seems probable that this factor plays a larger part in the pathogenesis of chest infection than is generally recognized. Whereas at times the mechanism of obstruction is based on a mucous plug arising from higher up the respiratory tract, at others it seems as though mucosal swelling in the smaller bronchi, or even partial stenosis resulting from past infection, is the precipitating factor. In a study on the pathology of chronic bronchitis, Reid (1954) has recently shown the gross alteration in architecture which takes place in the terminal parts of the air passages in advanced cases. She has also observed that the main abnormality in mild cases was hypertrophy of the mucus-secreting elements in the mucosa, and Oswald (1953) has drawn

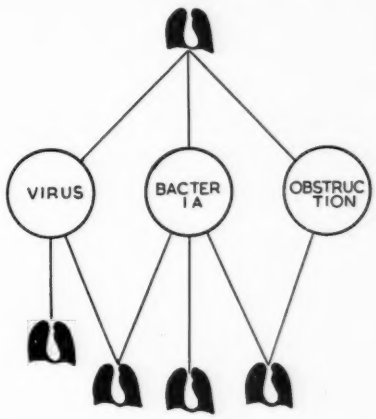

FIGURE VI

Diagram representing the way in which the three main ætiological factors are responsible, alone or in combination, for the pathogenesis of chest infections

attention to the clinical fact of hypersecretion of mucus in those who suffer from this condition. Such features are often more fundamental in the production of inflammation of the parenchyma than the presence in the respiratory tract of virus or bacteria.

While we await further elucidation of the relative importance of such factors, the energetic use of postural drainage, breathing exercises, and if necessary bronchoscopy, would appear to be a more rational form of therapy than complete reliance on chemotherapy.

SUMMARY

The present classification of pneumonia inadequately describes the ætiology and pathogenesis of this group of chest infections.

A study has been made of 22 patients from whose sputum a *Staph. aureus* was grown. In this series, the chest infection caused by the combination of influenza virus and staphylococcus is contrasted with the pulmonary lesions which are part of staphylococcal

septicæmia; a third group suggests that an ætiological agent of importance is bronchial obstruction.

It is concluded that factors other than the presence of a particular kind of bacterium are responsible for the development and the characteristics of pneumonia. In addition to the fulminating type of pneumonia referred to in this paper, the influenza virus is known to play a part in other types of what are regarded as "bacterial" pneumonias; it seems certain that the full range of activity of this and other viruses in pneumonia is at present unknown. Similarly, while the part played by bronchial obstruction is recognized as a factor of importance in a few cases, it is probably a feature of many.

ACKNOWLEDGEMENTS

I wish to acknowledge the assistance given to me by those Honorary Physicians of the Royal Perth Hospital who have allowed me to study their cases, and to thank Dr. Eric G. Saint, Director of the Clinical Research Unit, for his encouragement.

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Proceedings of the Royal Australasian College of Physicians

ORDINARY MEETING, 1954

THE Ordinary Meeting of the College in 1954 was held at Sydney from October 13 to 16. It was attended by 162 Fellows and Members. Professor R. McWhirter, Professor of Medical Radiology in the University of Edinburgh, was present at the meeting and participated in a clinical meeting at Sydney Hospital. The President, Dr. C. G. McDonald, was in the chair.

SCIENTIFIC SESSIONS

Two scientific sessions were held in the Stawell Hall of the College.

DOUGLAS STUCKEY (New South Wales) opened the first scientific session with a paper entitled "Cardiac Pain in Association with Mitral Stenosis and Congenital Heart Disease". In this paper the incidence of classical *angina pectoris* (angina of effort) in young subjects suffering from rheumatic or congenital heart disease was investigated. Only men aged under thirty-five years and women aged under thirty-seven years were selected. These age limits, based on a statistical study of 1600 cases of uncomplicated coronary heart disease, represented the ages below which coronary artery disease of clinical importance was least likely to occur. Among 400 patients suffering from mitral stenosis, *angina pectoris* occurred in 21 young subjects (5%), of whom seven had severe pulmonary hypertension and the remainder uncomplicated severe mitral stenosis. Females predominated in the proportion of 4·7 : 1·0. There were no such cases among 100 patients suffering from aortic incompetence or among 40 patients with pure mitral incompetence. Of 471 patients with congenital heart disease, *angina pectoris* occurred in 20 under the age limits mentioned (4·2%). These fell into the following three groups: aortic stenosis, severe pulmonary stenosis, and pulmonary hypertension, either primary or complicating a shunt. Exercise tests were carried out in 33 cases. Changes in the electrocardiogram on exercise indicating myocardial ischæmia were found in 12 of 18 patients with *angina pectoris*, and slight changes in only two of 15 controls. The changes were best seen in left ventricular surface leads, even in the presence of predominant right ventricular hypertrophy. Trinitrin given in full dosage before the test did not prevent ischæmic changes in the electrocardiogram in two cases. There was no evidence of coronary artery disease at post-mortem examination on 11 of these patients with mitral stenosis or congenital heart disease who had suffered from *angina pectoris* during life. All these patients had a mechanical obstruction to the circulation of the blood, either organic valvular stenosis or obstruction in the pulmonary circuit associated with pulmonary hypertension. The cardiac output at rest was low as estimated clinically and confirmed at cardiac catheterization in 25 cases, and it was calculated that this did not increase normally on exertion. The mechanism was quite different from that of classical *angina pectoris* occurring in older patients with coronary artery disease. It was suggested that a low relatively fixed cardiac output was the basic fault, and this was responsible for inadequate coronary blood flow during exercise, which produced relative myocardial ischæmia and cardiac pain.

ROBERT SOUTHBY (Victoria), in presenting a contribution entitled "Achlorhydria in Children—Some Observations on the Familial Aspect", submitted the following main points for consideration: (i) Achlorhydria in children was recognizable as a clinical entity. (ii) Those children presented in four groups: (*a*) a diarrhœal group, (*b*) an anæmic group, (*c*) a colitic group, (*d*) an allergic group. (iii) There was a definite familial incidence of achlorhydria in children. (iv) One small group, responding to folic acid therapy, might prove to be potential candidates for pernicious anæmia later.

The following contributions were also presented: "An Analysis of 70 Cases of Hiatal Hernia with Particular Reference to Symptomatology and Diagnosis", by GEORGE HALL and NOEL NEWTON, F.R.A.C.S. (New South Wales); "An Experimental Study of Hypertensive Encephalopathy", by F. B. BYROM (New South Wales); "Early Medical Works in Australian Collections", by EDWARD FORD (New South Wales); "Some Observations on Œsophageal Reflux", by S. J. M. GOULSTON (New South Wales); "Some Observations on Ulcerative Colitis", by A. W. MORROW (New South Wales); "The Effects of Hypocalcæmia on the Central Nervous System: Experimental Studies", by L. C. A. WATSON (New South Wales—from the Clinical Research Unit, Royal Prince Alfred Hospital).

CLINICAL MEETINGS

Clinical meetings were held at the Royal North Shore Hospital and at Sydney Hospital. The following demonstrations were given: "Gerstmann's Parietal Lobe Syndrome", George Selby; "Dermatomyositis", C. B. Hudson; "Myelofibrosis", S. E. L. Stening; "Thrombocytopenia", A. E. McGuinness; "Unusual Mediastinal Tumour", K. B. Noad; "Acquired Hæmolytic Anæmia", Innes Brodziak; "Phæochromocytoma", John Beveridge; "Elliptocytosis"; "Torulosis", Bruce Geddes.

MEMBERSHIP

Admission of Members. An examination for Membership was held in Australia in September and October, 1954. The following successful candidates were admitted to Membership by the President at the Council Meeting on October 13: C. H. Campbell of Queensland; B. S. Hartnett, A. P. Millar and J. D. Murphy of New South Wales; I. H. W. Anderson, J. R. E. Fraser, G. J. Groves, W. H. Kitchen, Graeme Larkins, D. H. Meyers, and M. G. Whiteside of Victoria; T. S. Kirkland of Tasmania. J. F. Funder of Victoria was admitted to Membership under the provisions of Article 37.

Honours. Honours have been bestowed by Her Majesty the Queen upon the following Fellows of the College: Sir Archibald Collins, Knight Bachelor; W. E. L. H. Crowther, Commander of the Order of the British Empire.

Obituary. The Council records with regret the death of W. T. Nelson of Sydney, of F. V. G. Scholes of Melbourne, and of T. W. J. Johnson of Auckland, who were Fellows of the College.

GENERAL

Scholarships. The Travelling Scholarship in Medicine for 1955 was awarded to G. H. Neilson of Queensland. The Wunderly Travelling Scholarship in Thoracic Disease for 1955 was awarded to H. P. B. Harvey of New South Wales.

Representatives of the College. J. G. Hayden was reappointed to represent the College on the National Health and Medical Research Council, and also to the Electoral College of St. Vincent's Hospital, Melbourne.

WHOLLY SET UP AND PRINTED IN AUSTRALIA
BY AUSTRALASIAN MEDICAL PUBLISHING
COMPANY, LIMITED, SEAMER STREET, GLEBE,
NEW SOUTH WALES.

VOLUME 4 FEBRUARY 1955 NUMBER 1

CONTENTS